Gastrocnemius medialis muscle-tendon properties and their relationship with power and reactive strength in elite youth soccer players

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A thesis submitted in partial fulfilment of the requirements of Liverpool John Moores University for the degree of Doctor of Philosophy

Name(s) of Collaborating Establishment(s): Liverpool Football Club

March 2021

Contents

Abstract

There are several key physical performance indicators (KPIs) in soccer, and these include lower limb power and reactive strength, which can be evaluated using a variety of jump assessments, e.g. countermovement jumps (CMJs) and drop jumps (DJs). These KPIs are thought to be underpinned by the physiological characteristics of the respective lower limb muscle-tendon units (MTUs). However, the influence of the gastrocnemius medialis (GM) MTU on power and reactive strength in elite youth soccer players (ESP) is currently unknown.

Before assessing MTU properties and KPIs in ESP, it is important to understand the reproducibility of the respective assessment methods. In chapter 3, the aim was to identify the between-session reliability of all methodological techniques involved in the subsequent chapters. For this, 12 physically active, healthy, young men were recruited, the findings demonstrated all morphological properties of the GM muscle and GM tendon could be measured with high levels of reproducibility (coefficient of variation (CV)=1.2-6.6%; mean bias ratio (MBR)=1.00-1.09, ratio limits of agreement (RLoA)=1.04-1.22). Mechanical and material properties of the GM tendon could also be measured reliably (CV=2.6-17.3%; MBR=1.00-1.13, RLoA=1.08-1.80), however, the reproducibility of these techniques was slightly lower than for the morphological properties. Furthermore, a simplified method for calculating GM tendon mechanical/material properties proved reliable and agreed with the more comprehensive method, when assessed in 13 ESP and 12 non-athletes. This provides researchers/practitioners with a valid, reliable, and efficient method to monitor GM MTU properties when time is limited, e.g. when working with elite athletes. During jump-based

assessments, peak power was measured more reliably during bilateral than unilateral CMJ, while reactive strength temporal variables during a DJ were more reliable than kinetic parameters.

The second experimental study (chapter 4) aimed to determine whether the physiological characteristics of the GM muscle, as well as lower-limb power and reactive strength (the ability to develop maximal forces in minimal time while changing from an eccentric to concentric contraction) during CMJ and DJ, differed between ESP and recreationally active control participants (CON). A second aim was to investigate the relationship between GM muscle properties with lower limb power and reactive strength. ESP (17.4 ± 2.5°) displayed a larger GM fascicle pennation angle (θ_p) compared to CON (14.3 ± 1.2°, P<0.001), while projectile range and horizontal peak power during bilateral and unilateral horizontal CMJ were greater in the ESP. Furthermore, GM θ_p and cross-sectional area correlated with power generated in the vertical and horizontal plane of motion during unilateral vertical and bilateral horizontal CMJ, respectively. Perhaps surprisingly, GM muscle fascicle length and muscle volume both correlated inversely with power during bilateral vertical and horizontal jumps, although this may represent the differing biomechanical demands between unilateral and bilateral jumping tasks.

The third experimental study (chapter 5) aimed to determine whether the mechanical and material properties of the GM tendon differed between ESP and CON, and whether GM tendon properties were related to lower limb power and reactive strength during CMJs and DJ. Tendon properties did not differ between ESP and CON but tendon elongation and strain correlated inversely with power during unilateral vertical CMJ, whilst higher force and vertical stiffness outputs were achieved during a DJ by stronger individuals with stiffer and larger GM tendons. Together with the findings from chapter 4, the results from chapter 5

suggest that specific physiological characteristics of the GM MTU could be deemed favourable for lower limb power and reactive strength capabilities. Through identifying (and perhaps developing) these characteristics in ESP, such KPIs in soccer players may be improved.

The purpose of the fourth and final experimental study (chapter 6) was to investigate the effects of a 12-week in-season soccer training programme on GM MTU properties and jump performance in 14 ESP. Post-training, GM θ_p (pre-training: 18.0 ± 2.5 °; post-training: 17.2 ± 2.6 °; P=0.038), maximal tendon elongation (pre-training: 2.68 ± 0.58 cm; post-training: 2.30 ± 0.46 cm; P=0.019) and maximal tendon strain (pre-training: 12.8 ± 2.4 %; post-training: 11.1 ± 2.5 %; P=0.019) all reduced. Furthermore, projectile range during a unilateral horizontal CMJ increased from pre- to post-training (pre-training: 0.93 ± 0.15 m; post-training: 1.07 ± 0.12 m; P=0.001) but no other jump metrics changed. The physiological reasons for these changes are unclear but it is plausible that GM θ_p decreased to facilitate a faster shortening of the overall muscle in response to pitch-based training. Alternatively, it may have occurred in response to the limited progressive overload applied to the plantar flexors during in-season training (potentially leading to a detraining effect). Further, the reductions in tendon elongation and strain may have been due to a change in the material properties of the tendon (e.g. increased density of collagen fibrils) that did not quite translate to changes in stiffness or modulus. Crucially, however, these changes did not correlate (neither individually, nor in combination) with the change in projectile range during a unilateral horizontal CMJ, thus suggesting they did not contribute to this increase in jump performance. These results demonstrate that a 12-week period of in-season soccer training is not a sufficient chronic stimulus to elicit meaningful adaptation of the GM MTU to cause significant improvements in power and reactive strength during different jump-based assessments.

In summary, this thesis demonstrates that the reproducibility of the techniques used to characterise GM MTU properties is generally acceptable to very good. Furthermore, specific GM MTU properties are related to lower limb power and reactive strength. Moreover, this thesis demonstrates that a three-month period of in-season soccer training is insufficient to elicit meaningful GM MTU adaptations required to improve physical performance. Therefore, whilst multiple factors contribute to influencing KPIs in ESP, this thesis presents novel information regarding the GM MTU and highlights potential physiological determinants that can be developed in youth soccer players.

Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Acknowledgements

I would like to thank everyone who has been a part of my journey and has helped make this PhD possible.

Firstly I would like to thank my director of studies, Dr Rob Erskine. I could not have asked for a better supervisor to support me through this journey. This process has taken longer than I imagined, but the confidence and faith that you put in me has driven me forwards through difficult moments. Your passion, knowledge and expertise for this project have been priceless and the support you provided was far beyond my expectations. You have pushed me to complete this thesis to the best of my ability and I hope that we can continue to collaborate on applied research in years to come.

Secondly, Professor Barry Drust. I am honoured to have been under your supervision for the past four and a half years. You helped keep everything in perspective and enabled me to see the bigger picture, even in some of the most challenging times. Your guidance and advice throughout has been invaluable, you have helped me develop as a researcher, practitioner and person, for that I am forever grateful.

To my other supervisors, Professor Olivier Seynnes and Dr Conall Murtagh, it has been a privilege to have your input on this project. Thank you for the time and effort you have spent helping to shape this thesis, I am proud to have your contribution a part of my work.

This experience would not have been possible without Liverpool Football Club and my former colleagues. In particular, thanks to Dr Jack Ade for providing me with this opportunity and your constant support and guidance since I joined LFC. I have been fortunate enough to learn from and work alongside some outstanding practitioners and people who have supported me through this process and allowed me to develop professionally. Amongst many I would like to thank Ollie Morgan, Adam Rowan, John Hill, Tom Maynard, Adam

Sullivan and Frazer Findlay. A very special mention goes to Craig Burnett. I'm grateful that we could start this journey together and share our experiences along the way, both good and bad. This process would not have been the same without your support and I am truly thankful for having you as my friend from the very first day.

To Mum and Dad, I cannot thank you both enough for everything you have done for me. I would not be in this position without your unconditional love and support, I cannot emphasise just how important that has been to me throughout this process. I hope that I have done you proud.

Finally to my fiancée Meg Hopkinson, I dedicate this PhD to you. This has been an incredibly challenging four and a half years for me and would not have been possible without having you by my side. You have made sacrifices and put your life on hold to allow me to pursue my passion in what has been a time-consuming and overwhelming journey. I thank you for all your support and everything you have done for us, I am looking forward to the next chapter of our lives together.

List of Abbreviations

ACSA; anatomical cross sectional area

CE; contractile element

CMJ; countermovement jump

CON; control participants

CSA; cross sectional area

CV; coefficient of variation

DF; dorsiflexion

DJ; drop jump

ESP; elite youth soccer players

EFOV; extended field of view

F_l; fascicle length

GL; gastrocnemius lateralis

GM; gastrocnemius medialis

GRF; ground reaction force

HCMJ; horizontal countermovement jump

ICC; intraclass correlation coefficient

IKD; isokinetic dynamometer

KPI; key performance indicator

MBR; mean bias ratio

MT; muscle thickness

MTU; muscle tendon unit

MTJ; myotendinous junction

MVC; maximal voluntary contraction

OTJ; osteotendinous junction

PCSA; physiological cross sectional area

PF; plantar flexion

RLoA; ratio limits of agreement

RSI; reactive strength index

S&C; strength and conditioning

SEE; series elastic element

TA; tibialis anterior

TE; typical error

VCMJ; vertical countermovement jump

VL; vastus lateralis

V_m; muscle volume

θ_p; fascicle pennation angle

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Chapter One

General Introduction

1.1 Introduction

Professional soccer clubs in England typically have an academy structure associated with their organisation that aims to develop youth players capable of representing their senior team. These academies often utilize a multi-disciplinary approach to developing their youth players, where a four corner model is frequently adopted (Towlson *et al.*, 2019; Kelly *et al.*, 2020). This model provides a holistic framework for the assessment and development of an individual's technical/tactical, physical, psychological, and social attributes. Multi-disciplinary teams are employed to work collaboratively, but each with the dedicated responsibility for one of these areas, and a strength and conditioning (S&C) coach is accountable for the physical aspects. Within their remit, S&C practitioners are required to evaluate the physical demands of sporting competition and provide appropriate interventions that are capable of developing key physical qualities underpinning performance.

Time-motion analysis can provide the practitioner with information regarding the movement demands of competition, which has been the focus of previous research within soccer (Di Salvo *et al.*, 2010; Faude, Koch and Meyer, 2012; Murtagh *et al.*, 2019). Through identifying frequent movement patterns and key performance indicators (KPIs), physical testing batteries can be designed and implemented to quantify the influential physical qualities of such tasks. Previous research has utilised this approach to highlight the importance of maximal accelerations from both static and leading starts, along with maximal velocity sprinting, albeit a less frequent occurrence (Faude, Koch and Meyer, 2012; Murtagh *et al.*, 2019). Furthermore, when assessed in isolation, sprinting performance over 10 m and 20 m has proven to be a differentiating capability between elite and non-elite youth soccer players (Emmonds *et al.*, 2016; Murtagh, Brownlee, *et al.*, 2018). Therefore, whilst performance in these tasks can be influenced by technical parameters, practitioners should aim to identify and quantify the key physical qualities underpinning acceleration and maximal velocity sprinting.

Biomechanical analysis of these tasks has demonstrated the distinctly different kinetic requirements for each phase, which are influenced by different physical qualities. Performance in acceleration tasks, which commonly occur in soccer, is largely influenced by lower limb power, whilst reactive strength capabilities are considered a determining factor for maximal velocity performance (Morin, Edouard and Samozino, 2011; Buchheit *et al.*, 2014; Colyer *et al.*, 2018; Meyers *et al.*, 2019; Douglas *et al.*, 2020). Therefore, the role of a soccer-based S&C practitioner should be, in part, to effectively quantify lower body power and reactive strength as part of an individualised needs analysis of their athletes. Additionally, practitioners must understand the physiological characteristics that underpin these physical attributes to allow for evidence-based interventions to be applied, directed towards improving physical performance.

Lower body power can, however, be quantified using numerous methods, such as loaded and unloaded countermovement jumps, Olympic lifting derivatives and maximal effort static cycling pedalling (Samozino *et al.*, 2008; Suchomel *et al.*, 2014; Kordi *et al.*, 2017). Clearly there is no singular objective assessment of power, although practitioners may wish to consider the specificity of the method to the associated task. For example, the specific behaviour of muscle-tendon unit (MTU) during acceleration might best be replicated during an unloaded countermovement jump (Kurokawa, Fukunaga and Fukashiro, 2001; Kurokawa *et al.*, 2003; Aeles *et al.*, 2018). Similarly, acyclic or repeated effort jumping tasks may provide the most appropriate replication of MTU behaviour during maximal sprinting (Ishikawa, Niemelä and Komi, 2005; Lichtwark and Wilson, 2006; Sousa *et al.*, 2007; Farris and Sawicki, 2012). Furthermore, kinetic analysis has demonstrated that acceleration requires force production to be orientated horizontally to the ground, whilst maximal sprinting progresses to a vertical orientation of force (Morin, Edouard and Samozino, 2011; Nagahara *et al.*, 2018). Therefore, these jumping tasks may be most informative if performed in the

relative direction of appropriate force application. However, few studies have utilised these approaches to monitor power output in elite athletic populations.

Therefore, isolated assessments of power and reactive strength should seek to replicate the biomechanical and physiological demands associated with the task they underpin, in this instance, acceleration and sprinting. In addition, specific characteristics of the muscle and tendon are known to influence the function of a MTU (Kawakami and Fukunaga, 2006; Roberts, 2016). Consequently, it is essential that the physiological characteristics that underpin and influence performance in power and reactive strength assessments are identified. Through this understanding, S&C practitioners can make informed assessments of physical capabilities and provide evidence-based interventions to elicit favourable adaptation of the MTU. In turn, these adaptations should improve the physical qualities pertinent to the demands of soccer.

Recent research has focussed on muscle-tendon properties of the knee extensors MTU in athletic populations (Helland *et al.*, 2013; Mersmann *et al.*, 2017; Murtagh, Nulty, *et al.*, 2018; Murtagh, Stubbs, *et al.*, 2018). However, the importance of the plantar flexors, in particular the gastrocnemius medialis (GM), during acceleration and sprinting tasks has been reported previously (Hamner, Seth and Delp, 2010; Dorn, Schache and Pandy, 2012; Hamner and Delp, 2013; Lai *et al.*, 2016; Schache *et al.*, 2019). Therefore, the role of the plantar flexors in power production and reactive strength capabilities should also be investigated. Whilst the plantar flexors have received some attention within the literature (Wiesinger *et al.*, 2017; Pentidis *et al.*, 2019), their association with power and reactive strength is currently under-researched, particularly in soccer players, thus warranting in-depth investigation.

Furthermore, cross-sectional research has often been implemented to examine and characterise the differences in muscle-tendon physiology in a variety of athletic populations,

including soccer players (Magnusson *et al.*, 2001; Arampatzis *et al.*, 2007; Wiesinger *et al.*, 2016; Murtagh, Nulty, *et al.*, 2018; Murtagh, Stubbs, *et al.*, 2018), but no study has yet investigated differences in GM MTU properties between soccer players and controls. Differences in morphological and mechanical properties between athletic groups could indicate an adaptive response to sport-specific training, which subsequently augments sporting performance. Alternatively, it could be the cause of supplementary training interventions or a genetic predisposition. Muscles and tendons are known to have a different adaptation time-course and response depending on the training stimulus (Franchi *et al.*, 2014; Bohm, Mersmann and Arampatzis, 2015; Wiesinger *et al.*, 2015; Damas, Libardi and Ugrinowitsch, 2018; Wackerhage *et al.*, 2019). Similarly, differences are present between MTUs, whereby characteristics of one MTU are not representative of another (Stafilidis and Arampatzis, 2007; Kubo *et al.*, 2011). Given the unique nature of soccer demands, inferences should not be made from other MTU properties reported in different athletic populations with a primary determinant of speed or endurance. Thus further work is required to investigate the role of the GM MTU in the physical performance of elite youth soccer players.

1.2 Aims and Objectives

The aim of this thesis was to investigate the muscle-tendon properties of the GM MTU in a cohort of elite youth soccer players (ESP) and understand how these characteristics influence lower body power and reactive strength. Four main objectives were implemented to achieve this aim:

1) Determine the reproducibility of current assessment methods used to quantify: (i) lower body power and reactive strength capabilities; (ii) morphological characteristics of the GM; and (iii) mechanical and material properties of the GM tendon. This topic is explored in the work described in Chapter 3.

2) Identify if GM muscle characteristics or performance in jumping tasks could distinguish a group of ESP from control individuals (CON), and whether these properties were related to lower body power and reactive strength. This topic is investigated in the work described in Chapter 4.
3) Establish whether GM tendon properties differed between ESP and CON, and whether these properties were related to lower body power and reactive strength. This topic is studied in the work described in Chapter 5.
4) Determine the effect of 12 weeks' in-season soccer training on GM MTU properties and jump performance in ESP. This topic is explored in the work described in Chapter 6.

Chapter Two

The role of the gastrocnemius muscle-tendon unit in generating power in soccer: A narrative review of the literature

Abstract

Physical profiling, including countermovement jumps (CMJs) and drop jumps (DJs) are frequently used to identify determinants of sprinting performance, such as lower limb power and reactive strength, which are considered key physical performance indicators (KPIs) in soccer. These KPIs are thought to be underpinned by specific muscle-tendon unit (MTU) physiological characteristics, although certain MTUs may be more influential than others, depending on the KPI. The aim of this narrative review was to (i) critically appraise the literature concerning the role of the gastrocnemius medialis (GM) muscle-tendon unit (MTU) in determining lower limb power and reactive strength performance; and (ii) highlight areas of limited understanding and provide direction for future research. *In vivo* GM MTU analysis during CMJ and DJ assessments has demonstrated distinct behaviours of the contractile element (CE) and series elastic element (SEE), which may influence performance during those jumping tasks. The CE is primarily responsible for producing large forces, whilst the SEE is essential to storing and transferring this force quickly. However, the relationships between GM MTU properties and power and reactive strength are under-researched and not well understood. Therefore, future research should investigate if GM MTU morphological and mechanical/material properties are related to power and reactive strength (particularly in elite athlete groups), and whether these properties change in response to a period of soccer training. Through this knowledge and understanding, practitioners will be able to implement specific training interventions that are designed to elicit MTU adaptations and thus improve physical performance.

2.1 Importance of power and reactive strength in elite soccer

Competitive soccer is an intermittent sport, physically characterised by periods of low intensity activity, such as walking and jogging, interspersed by short periods of high-

intensity, explosive movements and actions (Stolen *et al.*, 2005). Whilst distances covered at high-speed and sprint velocities only constitute a small amount of total distance covered (Di Salvo *et al.*, 2007), researchers have suggested that these actions are key performance indicators (KPIs); being both decisive in competitive fixtures and within talent identification strategies (Faude, Koch and Meyer, 2012; Emmonds *et al.*, 2016; Murtagh, Brownlee, *et al.*, 2018).

Linear sprinting is a complex skill in itself, with constant changes in kinetic and kinematic demands (Nagahara, Naito, Morin, *et al.*, 2014; Nagahara *et al.*, 2018). A singular sprint often comprises several phases, which during competitive soccer, has typically been categorised as either acceleration (<10 m) or maximal velocity (>10 m) (Murtagh *et al.*, 2019). Kinetic and kinematic determinants of sprinting have received significant attention within the literature, frequently demonstrating the importance of an individual's ability to generate and orientate large horizontal propulsive forces, coupled with short contact times during acceleration (Hunter, Marshall and McNair, 2005; Lockie *et al.*, 2011; Morin, Edouard and Samozino, 2011; Morin *et al.*, 2012, 2015; Kawamori, Nosaka and Newton, 2013; Nagahara *et al.*, 2018). Given that power is the product of force and velocity, the requirement for expressing large forces in short time frames would suggest that power is a key determinant of acceleration. This suggestion is supported through studies demonstrating the importance of power for acceleration capabilities (Morin, Edouard and Samozino, 2011; Buchheit *et al.*, 2014; Colyer *et al.*, 2018).

As an individual transitions from the acceleration phase to the maximal velocity phase of sprinting, the kinetic demands are altered, with an increase in vertical force application, potentially facilitating the shorter contact times and greater flight times necessary to maintain running velocity (Lockie *et al.*, 2011). In conjunction with the kinetic determinants of sprinting, an increase in vertical stiffness at higher velocities has been reported previously

(Nagahara and Zushi, 2017; Meyers *et al.*, 2019; Douglas *et al.*, 2020). Vertical stiffness demonstrates the ability to generate large forces and resist deformation of the lower limb to reduce displacement of the centre of mass, which is underpinned by reactive strength capabilities (McMahon and Cheng, 1990; Douglas *et al.*, 2020). A stiffer system with greater reactive strength would be expected to either reduce ground contact time for a given amount of force production, or generate a larger propulsive force for a given contact time, thus improving sprint performance. Therefore, net lower limb mechanical power and reactive strength qualities should be considered two of the main influential parameters for sprinting performance, and in turn, considered KPIs for elite soccer.

2.2 The use of jumping tasks to assess power and reactive strength

Given the importance of power and reactive strength for sprinting performance in soccer, isolation of these key physical qualities is imperative to quantify and monitor the capabilities of soccer players. Jumping tasks, such as drop jumps (DJ) and countermovement jumps (CMJ), provide one method for isolating reactive strength and power, which have been regularly used within the literature (Samozino *et al.*, 2008; Maloney *et al.*, 2016; Murtagh *et al.*, 2017; Douglas *et al.*, 2020).

Vertical height achieved during a CMJ can be calculated using numerous methods, but previous investigations have recommended using take-off velocity as the most appropriate estimate, given it cannot be influenced by airborne technique (Moir, 2008). Velocity can be determined through numerical integration of acceleration, as per Newton's laws of motion (Blazevich, 2007), and acceleration can be calculated from ground reaction forces (GRF). A similar approach has been applied for horizontal CMJ utilising anterior-posterior and vertical GRF to calculate projectile range (Meylan *et al.*, 2012; Murtagh *et al.*, 2017). Projectile range would negate issues with airborne and landing techniques and provide improved quantification of jump performance (Meylan *et al.*, 2012). There is, however, a paucity of

data utilising this method for bilateral or unilateral jumps, which requires further investigation to corroborate previous information regarding the reliability of projectile range (Meylan *et al.*, 2012).

However, despite the importance of both force and velocity in identifying jump height, the use of this parameter to predict lower body power has been criticised previously (Morin *et al.*, 2019). Power output is largely influenced by body mass, countermovement depth and the distance lower limbs extend during push-off phase, which jump height does not account for (Samozino *et al.*, 2008; Markovic *et al.*, 2014). Therefore, despite previously reported positive correlations and predictive capabilities of jump height from power and vice versa (Meylan *et al.*, 2010; Amonette *et al.*, 2012; Markovic *et al.*, 2014; Murtagh *et al.*, 2017), jump height alone is not sufficient at identifying power output (Morin *et al.*, 2019). Instead, power should be measured directly using GRF, or via a computation method that does incorporate jump height, but is not the sole determinant (Samozino *et al.*, 2008).

Furthermore, specificity of the jumping task to identify power associated with sprinting demands could question the use of a bilateral vertical CMJ. Given that the demands of acceleration involve absorbing and generating force unilaterally to propel the body forwards, a unilateral jump might be considered a more appropriate assessment and provide a greater insight into function. Equally, due to the direction-specific kinetic demands that are present with acceleration (Nagahara *et al.*, 2018), a horizontally orientated CMJ could be considered a more informative assessment of power. Despite Murtagh *et al.*, (2017) demonstrating the ability of peak power during unilateral jumping tasks to differentiate elite youth soccer players from non-elite players, few studies have investigated horizontal power during bilateral or unilateral horizontal CMJs and this requires further investigation (Meylan *et al.*, 2010, 2012; Murtagh *et al.*, 2017).

As previously mentioned, lower limb power represents one key physical attribute for sprinting performance, reactive strength qualities characterize another and are typically assessed during a DJ (Flanagan and Comyns, 2008). The nature of a DJ exposes the individual to an acyclic movement where they must tolerate very large braking forces and transfer this into propulsive concentric forces (Flanagan and Comyns, 2008; Douglas *et al.*, 2018) (typically referred to as 'reactive strength' (Douglas *et al.*, 2018)), a physiological demand often expressed in sprinting (Nagahara *et al.*, 2018). Leg stiffness, the peak centre of mass displacement relative to the peak vertical GRF (Hobara *et al.*, 2011), and reactive strength index (RSI) are reflective of this physical requirement and have been used previously to monitor reactive strength qualities during a DJ (Douglas *et al.*, 2020). Whilst RSI and leg stiffness are closely related (Kipp *et al.*, 2018), both being influenced by contact time, RSI can also be influenced by flight time of the jump and can incur technical differences. Leg stiffness on the other hand is solely determined by kinetic parameters during ground contact, which could be a more informative representation of an individual's reactive strength capabilities. However, larger test-retest variability in leg stiffness has been reported previously (CV ~14.6%) in comparison to GRF variables (CV ~6.2%) and RSI (CV 4.2-9.0%, depending on drop height) (Feldmann *et al.*, 2012; Byrne *et al.*, 2017; Douglas *et al.*, 2018; Maloney, Richards and Fletcher, 2018). Due to the range in variability of these metrics, further research is required to compare the reproducibility between all parameters before applying one approach to assessing reactive strength. Regardless, RSI and leg stiffness are thought to be largely governed by behaviours and characteristics of the muscle-tendon unit (MTU) of the ankle. Therefore, it is important to assess these properties in isolation and understand how they might underpin performance during these tasks.

2.3 The role of the plantar flexor MTU in jump assessments

CMJ and DJ tasks require a significant contribution from the plantar flexors to centre of mass acceleration, leg stiffness and overall work produced during jumping tasks as demonstrated previously (Bobbert, Huijing and van Ingen Schenau, 1986b; Pandy and Zajac, 1991; Farley and Morgenroth, 1999; Kuitunen, Ogiso and Komi, 2011; Maloney *et al.*, 2016; Wade, Lichtwark and Farris, 2018; Kipp and Kim, 2020)**.** Typically during jumps, a proximal to distal coordination sequence occurs, which influences the timing of its contribution and requires large angular velocities around the ankle during late push-off phases (Bobbert and van Ingen Schenau, 1988; Pandy and Zajac, 1991; Kurokawa, Fukunaga and Fukashiro, 2001; Kurokawa *et al.*, 2003; Farris *et al.*, 2016).

Work generated by the ankle during jumping tasks has been explained previously using Hill-type models, separating the MTU into a contractile element (CE) and series elastic element (SEE), which reflect the skeletal muscle and attaching tendon, respectively (Bobbert, 2001; Farris *et al.*, 2016; Wade, Lichtwark and Farris, 2018). The contribution of each element can vary depending on the jumping task; energy conservation during cyclical actions, such as running or hopping, power amplification during static jumping, or power attenuation during landing tasks (Roberts and Azizi, 2011). During jumping tasks, energy is generated at the ankle via three mechanisms; contractile force; energy stored and released via tendons; and force transferred from the knee via the biarticular gastrocnemius (Bobbert, Huijing and van Ingen Schenau, 1986b). Few studies have directly compared the behaviour and contribution of the gastrocnemius and soleus during jumping tasks, however, Farris *et al.*, (2016) demonstrated the similarities in stretch and recoil behaviour between the two muscles during vertical jumping. Furthermore, Kipp and Kim, (2020) utilised modelling analyses to identify comparable contributions of the two muscles to vertical acceleration in late stage of jumping, where the ankle is typically responsible for producing work. Therefore, due to the similarity

in behaviour between the main plantar flexor muscles, along with the extended role of the gastrocnemius in jumping tasks (transference of force), this review will focus on the muscle-tendon characteristics of the gastrocnemius medialis (GM). Whilst the inclusion of the soleus would provide an improved understanding of triceps surae function, the quality of soleus morphological/architectural imaging using ultrasound is often too limited to provide meaningful information (which was confirmed in my own pilot testing).

GM behaviour and that of its respective MTU, is comparable during a CMJ as it is during a DJ (Kurokawa, Fukunaga and Fukashiro, 2001; Kurokawa *et al.*, 2003; Ishikawa, Niemelä and Komi, 2005; Farris *et al.*, 2016). During early stages of a CMJ, GM MTU length remains constant during the initial downward phase of movement, despite continual shortening of fascicles and increases in fascicle pennation angle (Kurokawa, Fukunaga and Fukashiro, 2001; Kurokawa *et al.*, 2003). Similarly, during DJ tasks, the GM displays fascicular shortening in the build up to, and immediately after, touch-down, although some studies have suggested they remain in an isometric state or even undergo a period of lengthening after initial shortening, which might demonstrate a dependency on intensity (Ishikawa, Niemelä and Komi, 2005; Sousa *et al.*, 2007; Aeles and Vanwanseele, 2019). Regardless, both behaviours occur in parallel to immediate tendon lengthening, which permits muscle fibres to operate close to their optimum length, generating large amounts of force to be stored and released as elastic energy (Kurokawa, Fukunaga and Fukashiro, 2001; Kurokawa *et al.*, 2003; Ishikawa, Niemelä and Komi, 2005; Farris *et al.*, 2016). Rapid tendinous shortening, exceeding the maximal shortening velocity of fascicles, occurs during the push-off stage and is primarily responsible for the change in GM MTU length, along with the plantar flexor moment, as the fascicles remain in a quasi-isometric condition (Kurokawa, Fukunaga and Fukashiro, 2001; Kurokawa *et al.*, 2003; Ishikawa, Niemelä and Komi, 2005; Sousa *et al.*, 2007).

Several authors have identified this ‘catapult’ effect as necessary to ensure high angular velocities and large plantar flexion moments occur during late stage push-off (Bobbert, Huijing and van Ingen Schenau, 1986a, 1986b; Kurokawa, Fukunaga and Fukashiro, 2001; Kurokawa *et al.*, 2003; Ishikawa, Niemelä and Komi, 2005). Comparable to the MTU behaviour observed in jumping, Werkhausen, Cronin, Albracht, Bojsen-Møller, *et al.*, (2019) demonstrated an increase in tendon recoil when running with load or at higher velocities despite no change in strain. Such observations occurred in conjunction with minimal changes to GM fascicle behaviour, which suggest that tendon recoil is essential to facilitate overall MTU shortening when increased mechanical work at the ankle is required.

Therefore, it would appear that the CE of the GM MTU is primarily responsible for producing large forces, whilst the SEE is required to satisfy the high velocity demands during jumping tasks. Both attributes are underpinned by the morphological and mechanical/material characteristics of the MTU. Through understanding how performance might be influenced by these characteristics, training interventions can be directed towards eliciting specific adaptation aimed at improving power and reactive strength qualities.

2.4 The GM MTU determinants of contractile force and velocity

As discussed previously, the ability to generate large forces quickly alongside the ability to store and release large forces would appear beneficial for lower limb power and reactive strength in soccer players. The primary function of the GM MTU during jumping tasks is to generate as much power as possible, which can be achieved via an uncoupling of muscle and tendon to generate large forces and utilise elastic energy to amplify net MTU power. Therefore, we will seek to discuss the various morphological and mechanical elements of the respective tissues, which underpin this function and how they may influence physical performance.

2.4.1 Morphological determinants

2.4.1.1 Muscle size

Muscle size is a key determinant of force generating capacity (Jones, Rutherford and Parker, 1989) and can be measured *in vivo* using multiple techniques (Bamman *et al.*, 2000). Four frequently assessed indices of size are; muscle thickness (MT) anatomical cross-sectional area (ACSA), physiological cross-sectional area (PCSA) and muscle volume (MV) (Narici, Landoni and Minetti, 1992; Kanehisa, Ikegawa and Fukunaga, 1994; Kawakami *et al.*, 1994; Fukunaga, Roy, *et al.*, 1996; Maganaris *et al.*, 2001; Morse *et al.*, 2005; Erskine *et al.*, 2009). Whilst these measurements use 'gold standard' or validated techniques, there are still some discrepancies between the different methods regarding their appropriateness when determining the physiological contribution of muscle 'size' to force generating capabilities (Bamman *et al.*, 2000; Fukunaga *et al.*, 2001; Morse *et al.*, 2005; Blazevich *et al.*, 2009).

MT is determined via the distance between the superficial and deep aponeuroses at a singular (sagittal) location in a 2D image, whilst ACSA is identified through the outline of a single transverse 2D image (Bamman *et al.*, 2000). Due to the time efficient nature of both techniques, they have been a frequently used method of quantifying muscle size in the literature (Kawakami *et al.*, 1995; Bamman *et al.*, 2000; Fukunaga *et al.*, 2001; Erskine, Fletcher and Folland, 2014). However, whilst ACSA is a useful measure in muscles with a parallel fibre orientation, where it slices fibres at a right angle to their longitudinal axes (thus representing the total CSAs of all fibres and the total number of sarcomeres arranged in parallel (Jones, Rutherford and Parker, 1989), it underestimates the total parallel-aligned sarcomeres in pennate-fibred muscles (Narici, Landoni and Minetti, 1992).

MV on the other hand, is determined via multiple ACSA images, multiplying the muscle length by the sum of each ACSA (Kawakami *et al.*, 1994; Fukunaga, Roy, *et al.*, 1996).

However, calculation of MV can be affected by the number of CSA images taken, as a larger number of images are required to provide a reduced level of variation (Lund *et al.*, 2002; Nordez *et al.*, 2009). As MV is not only influenced by parallel-aligned sarcomeres but also those aligned in series, it will not accurately reflect the true force generating capacity of the muscle (Erskine *et al.*, 2009). Instead, PCSA has been referred to as the 'gold standard' index of muscle size to explain force output (Blazevich *et al.*, 2009), and requires knowledge of MV and architecture (i.e. MV / fascicle length) (Fukunaga *et al.*, 1992, 2001; Fukunaga, Roy, *et al.*, 1996; Blazevich *et al.*, 2009). Through the inclusion of muscle fascicle length in the PCSA equation, a true representation of total contractile tissue can be estimated, which can represent the force generation capabilities of a muscle (Fukunaga *et al.*, 2001). Although PCSA appears to provide an enhanced assessment of muscle size, its determination can be time consuming and impractical with groups of elite athletes, when time is often a limiting factor.

Larger muscle sizes are typically found in populations of elite athletes, particularly those with a principle requirement for speed and power (Sugisaki *et al.*, 2018; Fukutani *et al.*, 2020; Kordi *et al.*, 2020; Miller *et al.*, 2020). Research investigating the influence of muscle size on performance in jumping tasks is limited and slightly conflicting (Earp *et al.*, 2010; Dobbs, Gill, *et al.*, 2015; Murtagh, Nulty, *et al.*, 2018). Murtagh, Nulty, *et al.*, (2018) found positive correlations between peak vertical power during a unilateral CMJ and MV and PCSA of the quadriceps femoris muscle, and vastus lateralis (VL) muscle thickness, in youth soccer players. However, earlier research found no relationships between VL muscle size/architecture and any GRF parameters across a range of horizontal and vertical jumps (Earp *et al.*, 2010; Dobbs, Nicholas, *et al.*, 2015). Instead, authors reported positive correlations between gastrocnemius lateralis (GL) thickness and GRF outputs during a horizontal DJ, along with positive correlations between GL muscle thickness and peak power

outputs during a squat jump, CMJ and DJ. Taken together, these findings could suggest greater quantities of contractile tissue are favourable for force and power output during jumping tasks, although further investigation is required to clarify muscle-specific relationships.

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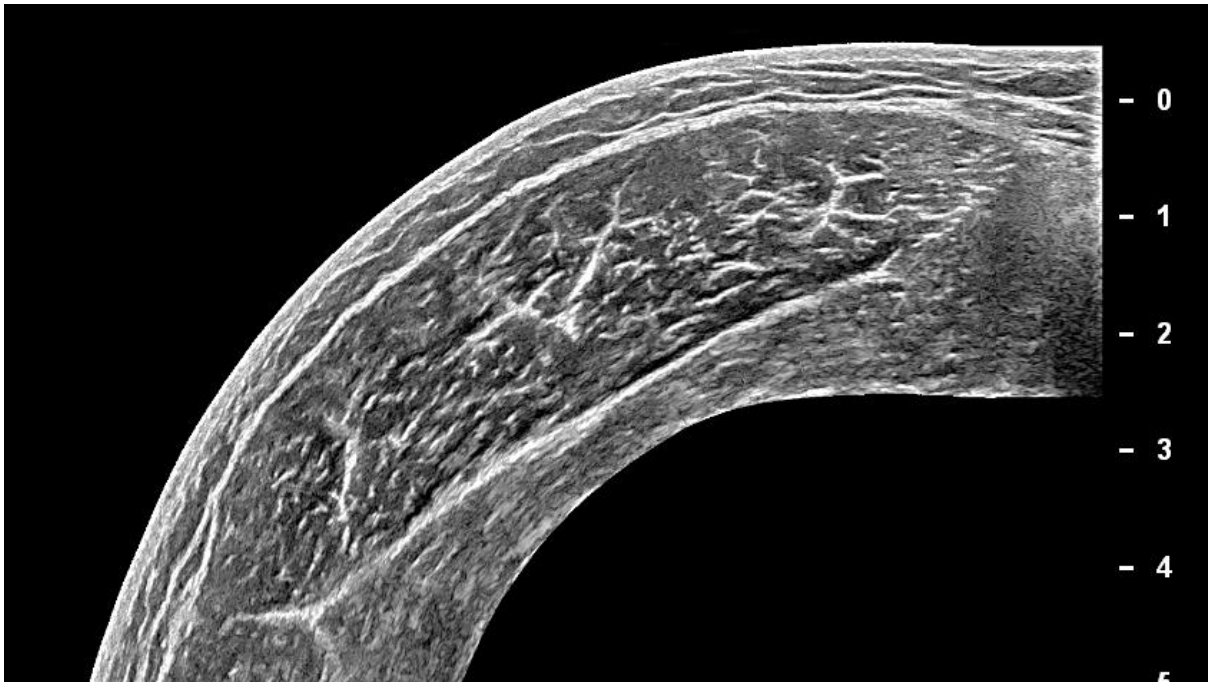

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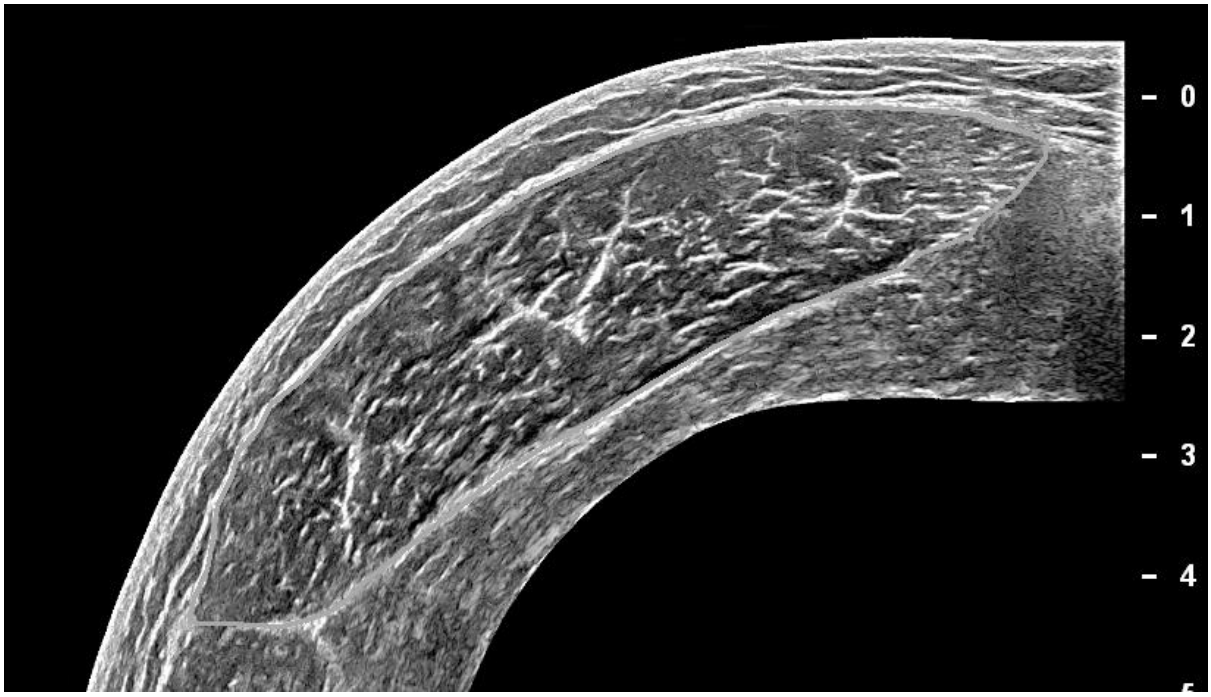

Figure 1. Representative ultrasound EFOV anatomical cross-sectional area (ACSA) image of the GM at 50% muscle length (A) and the GM ACSA outlined (B).

2.4.1.2 Muscle Fascicle Length

In addition to muscle size, skeletal muscle architecture has been the subject of significant research for several decades (Wickiewicz *et al.*, 1983; Kawakami, Abe and Fukunaga, 1993; Maganaris, 2003; Fukutani and Kurihara, 2015). Fascicle length represents the length of a bundle of muscle fibres, which in turn represents the number of in series sarcomeres (Wickiewicz *et al.*, 1983). Fascicle length can be determined by measuring the distance of one fascicle from the deep aponeurosis to superficial aponeurosis, as captured using ultrasound imaging (Rutherford and Jones, 1992; Kawakami, Abe and Fukunaga, 1993; Narici *et al.*, 1996). Depending on the muscle of interest, complete fascicle length often exceeds the field of view of a typical (4-6 cm wide) linear ultrasound transducer (Franchi *et al.*, 2018), so studies therefore used trigonometry (Kawakami *et al.*, 1995) or manually extrapolated (Erskine *et al.*, 2009) to estimate fascicle length (Blazevich, Gill and Zhou,

2006). However, the curvilinear nature of fascicle orientation and the inconsistent muscle thickness along the muscle length mean these methods can lead to error (Franchi *et al.*, 2018). Over the last decade, extended field of view (EFOV), or panoramic, ultrasound has been used to scan along the length of a skeletal muscle and create an image that displays the full length of a fascicle (Lin, Middleton and Teefey, 1999; Fornage *et al.*, 2000; Noorkoiv *et al.*, 2010). Studies have demonstrated the reproducibility of this technique along with its validity in comparison to the manual measurement of extracted fascicles from cadavers (Chleboun *et al.*, 2001; Noorkoiv *et al.*, 2010).

Longer muscle fascicles are thought to reflect a greater number of in series sarcomeres, which determines contractile speed, as it allows for a greater displacement in overall fascicle length (Spector *et al.*, 1980; Sacks and Roy, 1982; Wickiewicz *et al.*, 1983). Longer GM fascicles have been found in populations of elite sprinters compared with elite distance runners and untrained participants (Kumagai *et al.*, 2000; Abe *et al.*, 2001). Similarly, Aeles *et al.*, (2017) reported significant differences for GM fascicle length between elite track and field athletes (6.91 cm) with untrained age-matched individuals (6.12 cm) when both limbs were considered together. Only one study, to the author's knowledge, has examined the muscle architecture of the GM in elite soccer players, which reported shorter fascicles lengths in soccer players (5.6 cm) than elite swimmers (6.1 cm) (Kanehisa *et al.*, 2003). This difference was still observed when measured relative to lower leg length and could demonstrate the difference in physical demands between athletic groups (e.g. swimmers may be required to regularly generate greater power from the plantar flexors for maximum propulsion following push-off). From these studies, fascicle length may appear to be an important indicator of athletic success and favourable for athletes with a primary requirement for speed and power (i.e. track and field athletes), although it may also represent an adaptation that has occurred in response to sport-specific training. For team sports such as

soccer, which are not solely determined by speed and power, the importance of altered fascicle length is less clear and may be constrained by other physical demands (i.e. running economy).

This might, however, be surprising given the relatively slow shortening velocity of the GM fascicle during jumping tasks in comparison to tendon and overall MTU shortening. (Kurokawa, Fukunaga and Fukashiro, 2001; Kurokawa *et al.*, 2003; Ishikawa, Niemelä and Komi, 2005; Farris *et al.*, 2016). Few studies have investigated the relationship between GM fascicle length and performance in jumping tasks. Instead, positive relationships between VL fascicle length and vertical power during unilateral jumping tasks have been observed (Murtagh, Nulty, *et al.*, 2018). Interestingly, despite no direct correlation being found for GL fascicle length and jumping outputs, Earp *et al.*, (2010) found that the difference between CMJ and DJ performance could be predicted by GL fascicle length. However, this predictive ability was acknowledged as weak by the authors and their postulations remain speculative as there is no supporting evidence to suggest shorter fascicles can cope better with high eccentric forces than longer fascicles. The previously observed longer fascicle length in track and field populations could be the adaptation to a specific training stimulus (Blazevich *et al.*, 2003), however, the influence this would have on jumping performance is currently unknown. Given the lack of research investigating GM architecture and its influence on jumping performance, further research is required.

2.4.1.3 Muscle Fascicle Pennation Angle

Similar to muscle fascicle length, fascicle pennation angle can be identified using ultrasound imaging and represents the orientation of muscle fibres through the angle at which they attach to the aponeurosis. A larger pennation angle can allow for a greater number of sarcomeres to be structured in parallel and attach to the aponeurosis along its length (Wickiewicz *et al.*, 1983). An increased amount of contractile tissue attaching to the aponeurosis, particularly in

parallel, would provide a greater capacity to generate force (Wickiewicz *et al.*, 1983). Such a proposition has been supported in the literature, which has observed increases in maximal voluntary strength and muscle size with concurrent increases in pennation angle (Rutherford and Jones, 1992; Kawakami, Abe and Fukunaga, 1993; Aagaard *et al.*, 2001). However, in pennate muscles (such as the GM), the fascicle line of pull is not parallel to the tendon; instead, force resolved at the tendon occurs than an oblique transmission. Force generation is the result of fibre force and direction of force orientation; whereby a larger pennation angle can lead to a loss in transmitted force (Wakahara *et al.*, 2013). Therefore, it would appear that alterations in pennation angle should be considered a trade-off between hypertrophy of skeletal muscle and the direction of transmitted force.

It would be expected that if fascicle pennation angle remained constant during an isolated contraction, the velocity of contraction would also be impaired as the indirect line of pull would slow down muscle shortening and impair rapid force production (Maganaris, Baltzopoulos and Sargeant, 1998). Such an observation has been reported by Erskine, Fletcher and Folland, (2014) who found that changes in elbow flexor pennation angle, following a period of resistance training, correlated inversely with changes in normalised force at 150 ms during a rapid isometric contraction ($r = -0.362$, $P = 0.038$). This relationship was not observed at other time points (50ms and 100ms), potentially due to the lower reliability of these measures, or other intrinsic properties, such as neural drive or myosin heavy chain composition, influencing function during early phase contraction to a greater extent (Andersen and Aagaard, 2006). However, as discussed previously, the role of the gastrocnemius during jumping tasks does not appear to require fast fascicular contractile velocities.

Previous investigations have sought to understand the influence fascicle pennation angle might have on jump performance (Earp *et al.*, 2010; Dobbs, Nicholas, *et al.*, 2015; Murtagh,

Nulty, *et al.*, 2018). Murtagh, Nulty, *et al.*, (2018) found positive correlations between VL pennation angle with jump height and projectile range during unilateral vertical and horizontal CMJ respectively. Again, this contrasts with observations by Earp *et al.*, (2010), who found no relationship between VL fascicle pennation angle and height or power during a series of bilateral CMJ, DJ or squat jumps. Instead, the authors reported correlations between GL fascicle pennation angle with jump height and relative power output across all three jump types. Dobbs, *et al.*, (2015) on the other hand, reported correlations between GL and VL pennation angle with velocity parameters for a CMJ and squat jump. However, these studies have implemented different jump protocols with inconsistent assessment of jump outputs, using a variety of trained populations. The lack of consistency in methodology between studies can increase the complexity of forming meaningful conclusions or comparisons. Whilst these studies appear to support the notion that a larger fascicle pennation angle can increase the force generating ability during a CMJ, this research is sparse and requires further exploration before providing any definitive conclusions can be made.

Morphological adaptation of the GM has been investigated previously and it appears to respond positively to mechanical loading strategies, such as resistance training interventions (Geremia, Baroni, Lanferdini, *et al.*, 2018; Centner *et al.*, 2019; Gavanda *et al.*, 2020; Schoenfeld *et al.*, 2020). Increases in muscle size have been reported following a variety of loading regimes, which demonstrated that hypertrophy can occur across a 4-14 week intervention period (Geremia, Baroni, Lanferdini, *et al.*, 2018; Centner *et al.*, 2019; Gavanda *et al.*, 2020; Schoenfeld *et al.*, 2020). However, architectural adaptations of the GM following resistance training are less frequently observed, due to the limited number of studies that have investigated fascicle length or pennation angle. Given the population specific characteristics of GM architecture previously reported (Abe, Kumagai and Brechue, 2000; Kumagai *et al.*, 2000; Aeles *et al.*, 2017), it is crucial to know what GM architectural

characteristics soccer players possess and to understand how this might change in response to soccer training. However, there is currently know information on GM muscle characteristics in soccer players, so this warrants further investigation.

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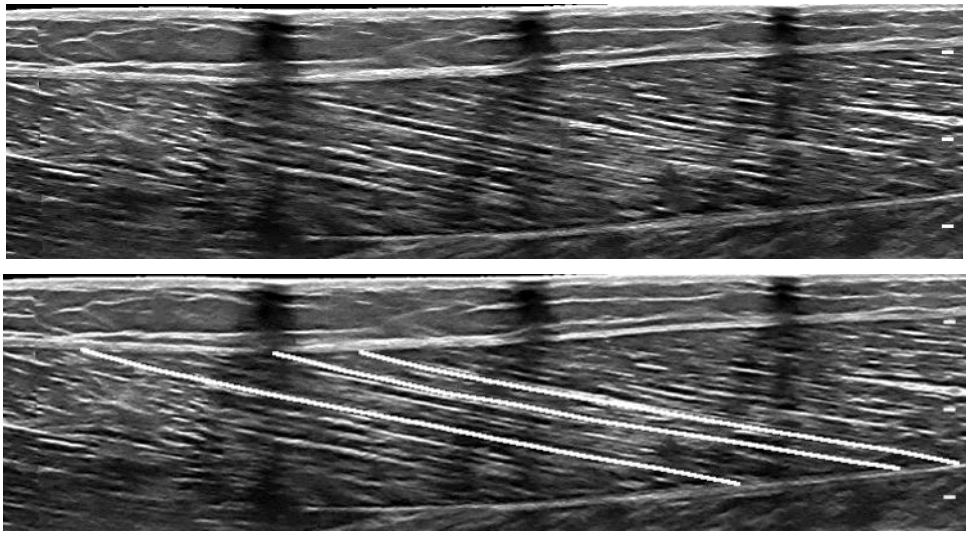

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Figure 2. Representative muscle architecture ultrasound EFOV images at 50% GM muscle length. The shadows represent the reference markers placed on the skin at 60% (left), 50% (middle) and 40% (right) of the GM length (**a**). Three fascicles have been traced from lower to upper aponeurosis in the same scan (**b**).

2.4.2 Tendon mechanical and material determinants

Tendon is a tissue with viscoelastic properties, consisting primarily of collagen. A tendon's principle function is threefold: 1) to transmit the force generated by the contractile tissue to bone, thus allowing skeletal movement; 2) to store and release elastic strain energy following a stretch during dynamic tasks; and 3) to uncouple deformation of muscle and MTU (Maganaris and Paul, 2002). Different aspects of a tendon are more responsible for these functions than others, whereby the aponeurosis primarily transmits contractile force that has been expressed laterally (through the extracellular matrix), whilst the tendon is responsible for the storage and release of energy (Magnusson *et al.*, 2003). Investigations into the mechanical properties of tendons have sought to better understand their influence on

function, athletic performance and adaptation to training. Advancements in technology, particularly high frequency ultrasound and MRI, together with isokinetic dynamometry, have allowed the mechanical and morphological characteristics of human tendons to be explored *in vivo*, overcoming previous limitations using cadaveric samples (Fukashiro *et al.*, 1995; Fukunaga, Ito, *et al.*, 1996; Smith, Young and Kearney, 1996).

2.4.2.1 Cross-Sectional Area (CSA)

Knowing the CSA and maximum force resolved at the tendon enables the stress (tendon force relative to its CSA to be calculated: the larger the tendon CSA, the lower the stress for a given force. A non-homogenous CSA is observed along the length of the Achilles tendon, where increases from proximal to distal aspects are reported (Magnusson and Kjaer, 2003; Bohm, Mersmann and Arampatzis, 2015; Intziegianni *et al.*, 2015; Holzer *et al.*, 2018). Given that tendons have a fracture stress value of approximately 100 MPa (Bennett *et al.*, 1986), the Achilles tendon generally has a low safety factor (2-3) (Magnusson *et al.*, 2001; Rosager *et al.*, 2002). A larger CSA would appear beneficial as it would allow the tendon to tolerate higher forces, possess a greater tensile strength and serve to increase this safety factor (Magnusson and Kjaer, 2003).

Cross-sectional research has explored morphological differences of the Achilles tendon in a variety of athletic populations (Rosager *et al.*, 2002; Magnusson and Kjaer, 2003; Wiesinger *et al.*, 2016). Investigators have frequently reported enlarged Achilles tendon CSA in running-based populations, leading to the suggestion that high habitual loading intensity or frequency, such as those imposed through locomotion, are a stimulant for tendon hypertrophy. Wiesinger *et al.*, (2016) suggested that, due to the independent CSA and stiffness observations between these populations, an increased CSA may occur to resist fatigue damage rather than assist the functional demands of stiffness. Furthermore, in sports that require the use of elastic energy, a lower ratio of muscle to tendon CSA was observed,

which might represent the functional demands of the tendon as a means to cope with the large forces generated by the contractile tissue (Wiesinger *et al.*, 2017). Interestingly, recent research by Monte and Zamparo, (2019) appear to support this suggestion, as they showed a relationship between Achilles tendon CSA and a variety of sprint performance markers including running velocity and power outputs. These findings suggest an increase in tissue quantity has a beneficial effect on performance, possibly by transmitting larger forces more effectively. Alternatively, this may be circumstantial, as the volume of high forces placed on the tendon during sprint training would likely stimulate an increase in tendon CSA.

However, to the best of the author's knowledge, no studies have investigated the relationship between Achilles tendon CSA and reactive strength or power qualities measured in isolation during jumping tasks. Without this information, it is difficult to conclude the importance of Achilles CSA on physical function, specifically power generation during rapid movement. Similarly, cross-sectional research only provides a snapshot of athletic populations at that moment in time and not information of training-induced adaptation. Thus, more research is required in this area.

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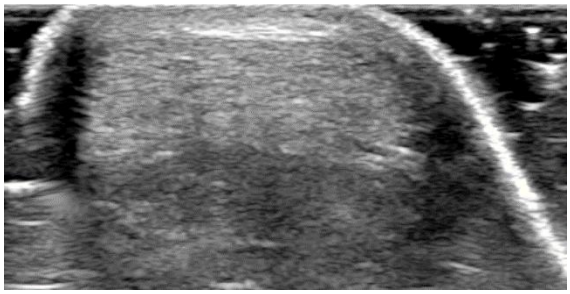

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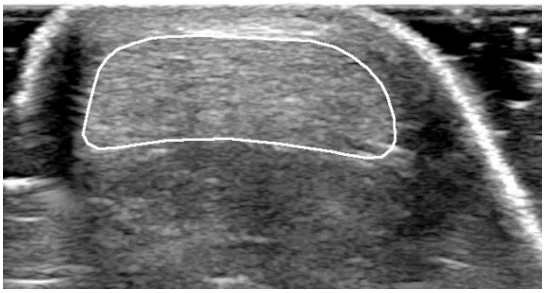

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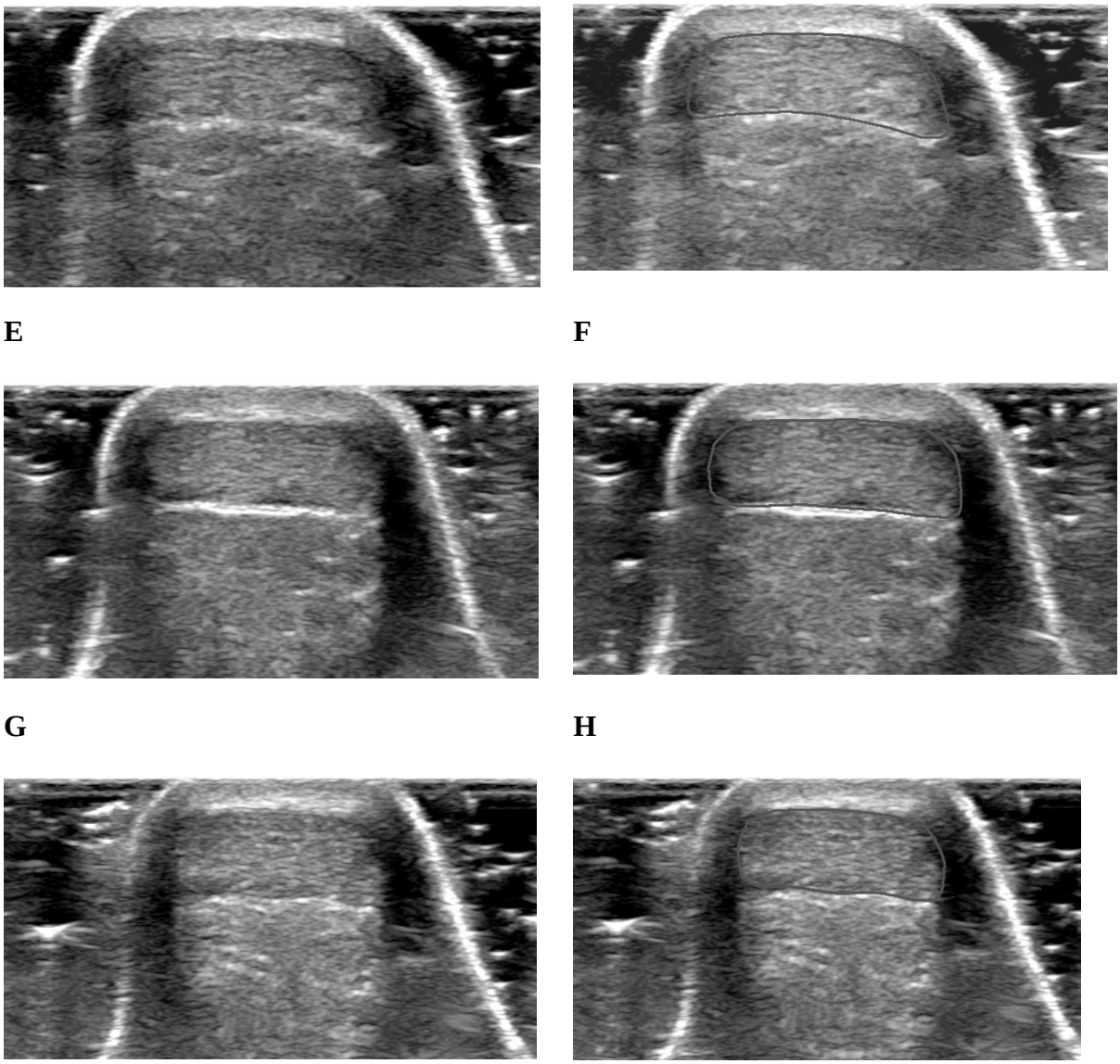


Figure 3. Ultrasound transverse cross sectional area images of the Achilles tendon where the outline has been highlighted at 1 cm (**a** and **b**), 2 cm (**c** and **d**), 3 cm (**e** and **f**) and 4 cm (**g** and **h**) proximal to the osteotendinous junction at the calcaneus.

2.4.2.2 Strain and Stiffness

In addition to morphology, exploring the force-elongation relationship has provided an insight into the viscoelastic properties of the tendon, which, when used in conjunction with its morphology, can provide information pertaining to its mechanical and material properties (Maganaris, Narici and Maffulli, 2008). Typically, four regions can be identified on a force-elongation curve; the initial region commonly known as the 'toe' region, which exhibits

elongation under low duress and is often influenced by the straightening of resting crimp without further deformation to collagen fibres. Of most interest is the second aspect, known as the 'elastic' or 'linear' region, whereby fibres still possess the elastic capabilities to return to their resting length, thus being viscoelastic. Additional loading stretches the straightened fibres to a point where some collagen fibres may start to break, although forces exerted during *in vivo* experiments may not reach the same linear region as exhibited during *in vitro* testing. Further elongation occurs in the plastic region, where greater breaking of fibres occurs, before complete failure and rupturing of a tendon in the final region (Maganaris, Narici and Maffulli, 2008).

As mentioned, the linear region is of particular interest, as it displays the elasticity of a tendon and represents its ability to store, release and transfer force, which is essential to athletic function. Maximal elongation during the linear region is used to calculate strain via changes in length from its resting position, whilst stiffness can be identified through the change in length relative to a change in force over a given range (Maganaris, Narici and Maffulli, 2008). Several authors have used this information to compare the mechanical properties between specific populations and associate characteristics with athletic performance (Arampatzis *et al.*, 2007; Kubo, Morimoto, Komuro, Yata, *et al.*, 2007; Fouré *et al.*, 2009; Wiesinger *et al.*, 2016; Pentidis *et al.*, 2020). However, despite the frequency of these measurements within the literature, significant methodological variances are present between studies, which can prevent direct comparison (Rosager *et al.*, 2002; Arampatzis *et al.*, 2007; Wiesinger *et al.*, 2016; Kubo *et al.*, 2017).

Several investigators have outlined key methodological considerations when utilising ultrasound to assess synonymous force and elongation data (Muramatsu, Muraoka, Takeshita, Kawakami, Hirano, Fukunaga, *et al.*, 2001; Arampatzis *et al.*, 2005; Maganaris, 2005; Fath *et al.*, 2010; Seynnes *et al.*, 2015). Of particular importance for the GM tendon, due to its length

exceeding the probes field of view, is accounting for distal tendinous displacement at the osteo-tendinous junction (OTJ) (Muramatsu, Muraoka, Takeshita, Kawakami, Hirano, Fukunaga, *et al.*, 2001; Maganaris, 2005). Any changes in joint angle rotation would cause a linear change in tendon displacement, (0.7 $mm \cdot {}^{\circ -1}$ for MTJ and 0.6 $mm \cdot {}^{\circ -1}$ for aponeurosis) (Fukunaga, Ito, *et al.*, 1996; De Monte *et al.*, 2006). If left unaccounted for, this could significantly overestimate tendon elongation (30-58% displacement of aponeurosis) (Magnusson *et al.*, 2001; Arampatzis, Monte and Karamanidis, 2008), which in turns affects values of strain, stiffness and Young's modulus (Magnusson *et al.*, 2001; Muramatsu, Muraoka, Takeshita, Kawakami, Hirano, Fukunaga, *et al.*, 2001; Arampatzis *et al.*, 2005; De Monte *et al.*, 2006).

Authors are in agreement that any motion at the ankle joint must be accounted for, however, methodological approaches to do so vary significantly. Maganaris, (2005) demonstrated that passive rotations of the ankle (a frequently implemented technique) occur around different axes of rotation to that during active conditions (maximal isometric contractions). Maganaris, (2005) concluded that the differing axes of rotation were the cause for observed variance in overall tendon displacement (passive rotations incurred ~10mm less displacement than active conditions). Therefore, tendon displacement at the OTJ must be accounted for during active conditions before being subtracted from overall elongation at the MTJ or aponeurosis (Maganaris, 2005). However, the use of this technique is limited within the literature (Kongsgaard *et al.*, 2011) and requires further investigation to determine the reproducibility of this method.

Whilst different methods employed within the literature to determine strain and stiffness prevent between-study comparison, several studies have investigated the mechanical properties of the GM tendon in a variety of athletic populations (Arampatzis *et al.*, 2007;

Wiesinger *et al.*, 2016; Kubo *et al.*, 2017). Strain and maximal elongation appear to be largely unaffected by athletic or training status. However, significant increases in elongation have been found following a period of detraining in both the plantar flexors and knee extensors, which resulted in the respective tendon elongating beyond pre-training or control values (Kubo, Ikebukuro, *et al.*, 2010; Kubo *et al.*, 2012). The authors suggested the change in elongation was attributed to changes in collagens fibre structure, following the cessation of resistance training and reduction in daily activity. In contrast, increases in GM tendon stiffness are frequently found following periods of high intensity resistance training (Arampatzis, Karamanidis and Albracht, 2007; Bohm, Mersmann and Arampatzis, 2015; Geremia, Baroni, Bobbert, *et al.*, 2018).

GM stiffness in various athletic populations has, however, shown contrasting findings. When normalised to body mass, Wiesinger *et al.*, (2016) reported greater GM tendon stiffness in ski jumpers, whose GM tendons undergo high loading intensities, when compared with water polo athletes and sedentary individuals. This could suggest that habitually high-loading sports cause the GM tendon to adapt by increasing stiffness. Similarly Arampatzis *et al.*, (2007) observed greater GM tendon stiffness in sprinters compared to a group of controls and endurance trained individuals. The authors chose to analyse the force-strain data, rather than elongation, in an attempt to normalise GM tendon length differences. This approach may explain why no other investigations have reported differences in GM tendon stiffness between groups of sprinters, endurance runners or controls (Kubo *et al.*, 2000, 2011, 2017; Stafilidis and Arampatzis, 2007).

In contrast to muscular adaptation, mechanical and morphological properties of the GM tendon appear to require a very high intensity loading strategy to elicit increases in stiffness and CSA (Arampatzis, Karamanidis and Albracht, 2007; Bohm *et al.*, 2014; Geremia, Baroni, Bobbert, *et al.*, 2018). Tendons are a non-force producing element that rely on the

muscular contractions to impose strain on them. It is this applied strain, in conjunction with the loading cycle, which largely dictates the adaptation outcome, which, if sufficient, can elicit increases in stiffness over an 8-14 week training period (Arampatzis, Karamanidis and Albracht, 2007; Bohm *et al.*, 2014; Geremia, Baroni, Bobbert, *et al.*, 2018). Optimal strain rates have been suggested to occur between 4.5-6.5% at a loading rate of 3 s (Arampatzis, Karamanidis and Albracht, 2007; Arampatzis *et al.*, 2010; Bohm *et al.*, 2014). Typically, strain rates less than this may not be adequate to invoke increases in stiffness (Arampatzis, Karamanidis and Albracht, 2007; Arampatzis *et al.*, 2010), although recent research has suggested that under hypoxic conditions, similar hypertrophic responses could achieve at a much lower training intensity (Centner *et al.*, 2019). Furthermore, continual progressive overload is essential to allow continued increases in Achilles tendon CSA and prevent a plateau from occurring, which has been reported previously (Epro *et al.*, 2017).

Furthermore, authors have sought to determine the influence tendon stiffness may have on performance in a variety of jumping tasks (Kubo *et al.*, 2000; Foure *et al.*, 2011; Abdelsattar, Konrad and Tilp, 2018; Murtagh, Stubbs, *et al.*, 2018). In particular, research investigating the patellar tendon has suggested a more compliant tendon is beneficial for jumping performance involving a countermovement (Kubo, Kawakami and Fukunaga, 1999; Kubo *et al.*, 2006; Murtagh, Stubbs, *et al.*, 2018). However, the relationship between GM tendon properties and jump performance are inconclusive due to contrasting findings (Burgess *et al.*, 2007; Kubo, Morimoto, Komuro, Tsunoda, *et al.*, 2007; Fouré *et al.*, 2009; Wu *et al.*, 2010; Abdelsattar, Konrad and Tilp, 2018; Pentidis *et al.*, 2020).

Several authors have demonstrated that positive correlations exist between GM tendon stiffness and jump performance, although this may depend on the type of jump employed (Burgess *et al.*, 2007; Kubo, Morimoto, Komuro, Tsunoda, *et al.*, 2007; Wu *et al.*, 2010; Pentidis *et al.*, 2020). A purely concentric jump, or DJ might rely on a faster transmission of

force to the bone, which can be facilitated by a stiffer tendon due to an increased rate of force development (Bojsen-Møller *et al.*, 2005; Burgess *et al.*, 2007; Kubo, Morimoto, Komuro, Yata, *et al.*, 2007a; Waugh *et al.*, 2013; Hirayama *et al.*, 2017; Abdelsattar, Konrad and Tilp, 2018). Similarly, for a stiffer tendon to obtain a given strain, the application of a larger force would be required, which in turn would enable the tendon to store and release more elastic energy. The relationship between tendon force and stiffness has been well documented, which could present a concurrent adaptation to training, whereby the intrinsic properties of a tendon change to maintain strain at higher forces (Arampatzis, Mersmann and Bohm, 2020). However, increases in GM tendon stiffness, following isometric training interventions, are not necessarily concurrent with improvements in jump height during a CMJ or DJ (Burgess *et al.*, 2007; Kubo, *et al.*, 2007a). Rather, jump heights can increase following plyometric training in the absence of changes to tendon stiffness (Kubo, *et al.*, 2007b; Fouré *et al.*, 2009).

Therefore, inconsistent findings between altered mechanical properties of the GM tendon and changes in jumping performance outputs have led to uncertainty in the relationship between the two variables and therefore require further investigation. Moreover, the majority of this research has utilised jump height as an indicator of performance which, as previously discussed, may not be an appropriate indicator of athletic performance. Instead research should be conducted investigating the influence mechanical properties have on the ability to generate power and reactive strength.

2.5 Summary

To conclude, lower limb power and reactive strength should be considered key physical qualities in elite soccer players and can be assessed in isolation during jumping-based tasks. Whilst recent research has sought to provide improved assessments of these physical qualities, more research is required to determine the reliability of such measures. Within

these assessments, the plantar flexors play a significant role in creating the large angular velocities required to accelerate the centre of mass vertically and to regulate lower limb stiffness during ground contact. This literature review has demonstrated the role of the GM muscle and attaching GM tendon within these tasks, where the contractile tissue is primarily responsible for producing large forces, whilst the tendinous tissue is essential to storing and transferring this force quickly. The ability of a MTU to function and improve performance during these tasks is underpinned by several key physiological characteristics, which have been outlined in this review. However, the influence these properties might have on power and reactive strength is currently unclear. Future research should focus on identifying these characteristics and determine if they are related to physical KPIs. Furthermore, there is a significant lack of research investigating the muscle-tendon properties of elite soccer players, which cannot be inferred by findings in other sporting populations. Therefore, research should address these gaps by identifying if specific morphological and mechanical properties exist in the MTUs of elite soccer players and, if so, how these might adapt in response to a period of in-season training. Current cross-sectional research does not provide enough insight into the adaptations that can be elicited through sport-specific training and whether or not these are favourable for sporting performance. Identifying favourable characteristics of a MTU and understanding how they respond to sport-specific practice, can inform practitioners when designing training interventions.

Chapter Three

Methodology and Reliability of Gastrocnemius Muscle-Tendon Unit Assessments and the Kinetic and Temporal Outputs During Jumping Tasks

Prelude

This chapter will provide a comprehensive overview of the methods used in the subsequent experimental chapters, including assessments of GM muscle-tendon properties, as well as markers of key physical performance indicators, such as power and reactive strength measured during different jumping tasks. Therefore, not only will this chapter describe the methodologies used in this thesis but it will highlight the reproducibility of those techniques, thus providing confidence in the data presented in the subsequent chapters, where any observed differences/changes can be attributed to physiological variance/change and not through measurement error.

Abstract

This study aimed to investigate (i) the agreement of a simplified method of assessing gastrocnemius medialis (GM) muscle-tendon unit (MTU) properties (Method 2) with a more comprehensive method (Method 1) using ultrasonography and isokinetic dynamometry; and (ii) the test-retest reproducibility of GM MTU neuromuscular assessments and different jump variables. Twenty-five physically active, healthy, males were recruited (aim 1) and a sub-sample (n=12) of those performed GM MTU assessments, bilateral and unilateral countermovement jumps (CMJ) and bilateral drop jumps (DJ) on two separate occasions (aim 2). Low coefficient of variation (CV=1.2-6.6%), excellent intra-class correlation coefficients (ICC=0.903-0.998, P<0.001) and minimal mean bias ratio with close ratio limits of agreement (MBR=1.00-1.09, RLoA=1.04-1.22) were observed for MTU morphological properties. However, tendon elongation, strain, stress, stiffness and modulus displayed larger variance (CV=2.58-17.3%; MBR=1.00-1.13, RLoA=1.08-1.80). Method 2 consistently underestimated Method 1 regarding tendon stiffness and Young's modulus, but overestimated tendon strain and maximal elongation (MBR=0.81 and 1.23, respectively). Peak vertical and horizontal power were more reliable during bilateral CMJ (CV=8.04-9.94%; MBR=0.96-0.98; RLoA=1.26-1.39) than unilateral CMJ (CV=9.49-16.8%; MBR=0.98-0.99, RLoA=1.31-1.51). Temporal parameters (CV=5.35-7.98%; MBR=1.00-1.01: RLoA=1.18-1.40) were more reliable than kinetic outputs during DJ (CV=10.2-21.5%; MBR=1.01-1.03: RLoA=1.39-1.65). Morphological characteristics of the GM MTU are therefore highly reproducible but tendon mechanical/material properties less so. A simplified method for calculating GM tendon mechanical/material properties can be implemented reliably; although a correction factor must be applied to account for under-/overestimation. Peak power can be more reliably measured during bilateral than unilateral CMJ, while DJ temporal variables were more reliable than kinetic parameters.

3.1 Introduction

Physical profiling can help isolate and monitor specific qualities pertinent to sporting (e.g. soccer) performance. Jumping tasks are frequently used to identify prominent determinants of sprinting performance, such as lower limb vertical stiffness and power (Morin *et al.*, 2012; Nagahara and Zushi, 2017; Colyer *et al.*, 2018; Douglas *et al.*, 2020). However, when selecting methods of assessment, the reliability of that assessment is paramount, as poor reliability may lead to erroneous conclusions regarding between athlete comparisons and/or training/injury-induced changes in athlete performance.

Jump height or distance achieved can be calculated using numerous methods (Moir, 2008; Meylan *et al.*, 2012), with recent research proposing an improved method to calculate projectile range during horizontal jumps that negates technical differences (Meylan *et al.*, 2012). Performance in unilateral jumping tasks has shown a direction-specific relationship with quadriceps femoris muscle-tendon properties (Murtagh *et al.*, 2017; Murtagh, Stubbs, *et al.*, 2018), although limited reliability data exist for projectile range methods and thus requires further investigation. However, as previously mentioned, the physical characteristics expressed during the jump should be the focus of assessment rather than the distance obtained, nevertheless, the reliability of power calculated during countermovement jumps has been shown to differ according to the direction and specific phase of the jump (Meylan *et al.*, 2010). Similarly, limited data exist that compare vertical stiffness (i.e. the peak centre of mass displacement relative to the peak ground reaction force, GRF) and reactive strength index (RSI, the ratio of flight time to contact time) during a drop jump, despite their strong associations with sprinting performance (Kipp *et al.*, 2018; Maloney, Richards and Fletcher, 2018; Douglas *et al.*, 2020).

Neuromuscular characteristics of the quadriceps femoris are related to performance in jumping tasks (Murtagh, Nulty, *et al.*, 2018; Murtagh, Stubbs, *et al.*, 2018) but the

contribution of the gastrocnemius medialis (GM) muscle-tendon unit (MTU) is currently inconclusive, in part due to variance in methodological approaches (Kubo, *et al.*, 2007a; Kubo, *et al.*, 2007b; Fouré, Nordez and Cornu, 2010; Wu *et al.*, 2010; Hirayama *et al.*, 2017; Abdelsattar, Konrad and Tilp, 2018). GM MTU properties have been investigated in a variety of populations, although inconsistent reliability has been reported for muscle thickness (MT), fascicle length (L_f), fascicle pennation angle (θ_p), tendon length and tendon cross-sectional area (CSA) (Mohagheghi *et al.*, 2007; Dudley-Javoroski *et al.*, 2010; Fouré, Nordez and Cornu, 2010; Legerlotz, Smith and Hing, 2010; Raj, Bird and Shield, 2012; K. Kubo *et al.*, 2014; Weisskirchner *et al.*, 2015; Intziegianni *et al.*, 2015; Silbernagel *et al.*, 2016; McMahon, Turner and Comfort, 2016; Kruse, Stafilidis and Tilp, 2017; Brouwer *et al.*, 2018). This inconsistency could be due to different assessment techniques being used to measure GM MTU properties.

Furthermore, GM tendon stress, strain, stiffness and Young's modulus can be calculated using the GM's resting tendon length, maximal elongation, CSA and moment arm (Seynnes *et al.*, 2015; Maganaris *et al.*, 2017). Maximal GM tendon elongation requires knowledge of its displacement at proximal and distal insertions, whilst moment arm is needed to calculate force resolved at the tendon (Fukunaga, Ito, *et al.*, 1996; De Monte *et al.*, 2006; Arampatzis, Monte and Karamanidis, 2008; Fath *et al.*, 2010). There is, however, no consensus on how to account for either distal tendon displacement, or Achilles tendon moment arm *in vivo*, as various methods have been implemented previously, which display varying values of reliability (Kubo, Kanehisa and Fukunaga, 2005; Maganaris, 2005; Fath *et al.*, 2010; Fouré, Nordez and Cornu, 2010; Waugh *et al.*, 2012, 2014; Fletcher and Macintosh, 2018). When investigating MTU properties in elite athletes, time is often a limiting factor, thus simpler methods may need to be employed to save time. However, the validity and reliability of any

simplified method needs to be investigated to provide confidence in that method before it can be implemented.

Whilst reproducibility data exist in the literature for a significant number of muscle-tendon properties, the statistical analysis frequently employed is fairly rudimentary (e.g. paired *t*-tests, Pearson's correlations, etc.) and has received criticism (Altman and Bland, 1983; Bland and Altman, 1986; Nevill and Atkinson, 1997). Instead, ratio limits of agreement, which would determine whether heteroscedastic variability exists (whereby the variability between tests is dependent on the size of the parameter), has been suggested to provide a better indication of reproducibility (and agreement between two methods to calculate the same variable) compared to correlation coefficients, which simply demonstrate the relation between two sets of values (Nevill and Atkinson, 1997).

Considering the above, the aims of this study were threefold: firstly, to describe in detail the methodologies used in the subsequent experimental studies of this thesis; secondly to investigate the test-retest reproducibility of neuromuscular properties of the GM MTU and jump variables typically used to monitor athlete performance; and thirdly, to investigate the agreement between, and compare the reliability of, two different methods of characterising the GM muscle-tendon properties. The main objective was to address the inconsistencies and gaps within the literature by providing robust reliability estimates to help inform future research and practice. It was hypothesised that assessments of GM MTU morphology would demonstrate higher reproducibility than those of GM tendon mechanical/material properties. In addition, it was hypothesised that bilateral jump variables would be more reproducible than unilateral, potentially due to increased stability during those tasks.

3.2 Method

3.2.1 Participants

Twenty-five physically active, healthy males (age = 20.3 ± 2.8 years, height = 1.78 ± 0.69 m, body mass = 72.3 ± 6.6 kg; mean ± SD) volunteered to take part in this study. All participants were free from any lower limb injury or neuromuscular condition. Ethical approval was granted for this study by Liverpool John Moores University Research Ethics Committee and the study complied with the Declaration of Helsinki.

3.2.2 Experimental Design

To compare the comprehensive method (Method 1) of assessing GM MTU properties with a simplified method (Method 2), GM MTU properties were assessed during one data collection session following familiarisation to all procedures in a previous lab visit. To assess test-retest reliability of the GM MTU properties and jump assessments, a sub-sample of 12 participants attended the laboratory for a third time (2-7 days following the second visit), during which GM MTU properties were assessed for a second time. During both data collection sessions, participants performed at least three maximal voluntary isometric plantar flexion (PF) and dorsiflexion (DF) contractions (MVCs) whilst seated on an isokinetic dynamometer, using only their dominant (kicking) limb. Additional 6-s ramped plantar flexion contractions (6-s from rest to MVC) and passive ankle rotations were performed on the isokinetic dynamometer with an ultrasound probe positioned over the distal GM myotendinous junction (MTJ) and osteotendinous junction (OTJ) at the calcaneus. After this, participants were taken through a standardised warm up, comprising dynamic movements and stretches, along with three practice trials at each of the different jump modalities. Three trials of each jump

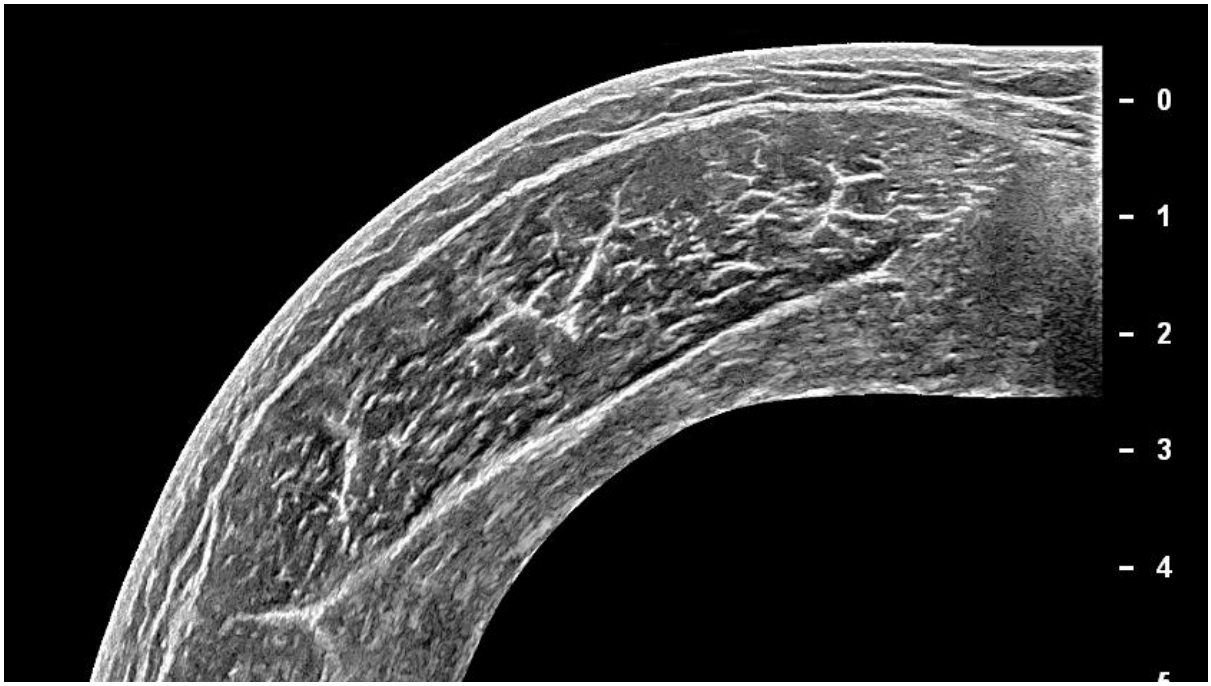
protocol were recorded using a force platform recording vertical, medio-lateral, and anterior-posterior GRF.

3.2.3 Data Acquisition

3.2.3.1 Muscle Size and Architecture

Participants lay relaxed in the prone position on a therapy bed during the GM ultrasound scans, with their foot fixed at an ankle angle of 0° (neutral). A 40 mm wide ultrasound probe (18-5 MHz), operating in real-time B Mode (Philips Epiq 7, Guildford, United Kingdom), was placed perpendicular to the skin in the sagittal plane to identify the distal and proximal MTJ of the GM. Muscle length was measured between the two points using an anthropometric tape measure (Seca 201, Liverpool, United Kingdom), where intervals at 20, 40, 50, 60 and 80% were marked along the muscle length. At each interval, the medial and lateral boarders of the GM were located with the ultrasound probe in the transverse plane, which allowed for the midpoint at each section to be identified and marked. Thin (2 mm wide) strips of surgical tape (3M Micropore, Bracknell, United Kingdom) were placed transversely at 50% muscle length to provide a visual reference point on the ultrasound image from the sagittal scan. With the ultrasound probe placed sagittally and perpendicular to the skin, and water-soluble gel aiding transmission, a panoramic (extended field of view, EFOV) image along the midline of the muscle was taken. With the ultrasound probe placed transversely, further panoramic images were taken from medial to lateral boarder at each of the aforementioned intervals. Minimal pressure was applied throughout the scans to avoid compression of soft tissue.

A



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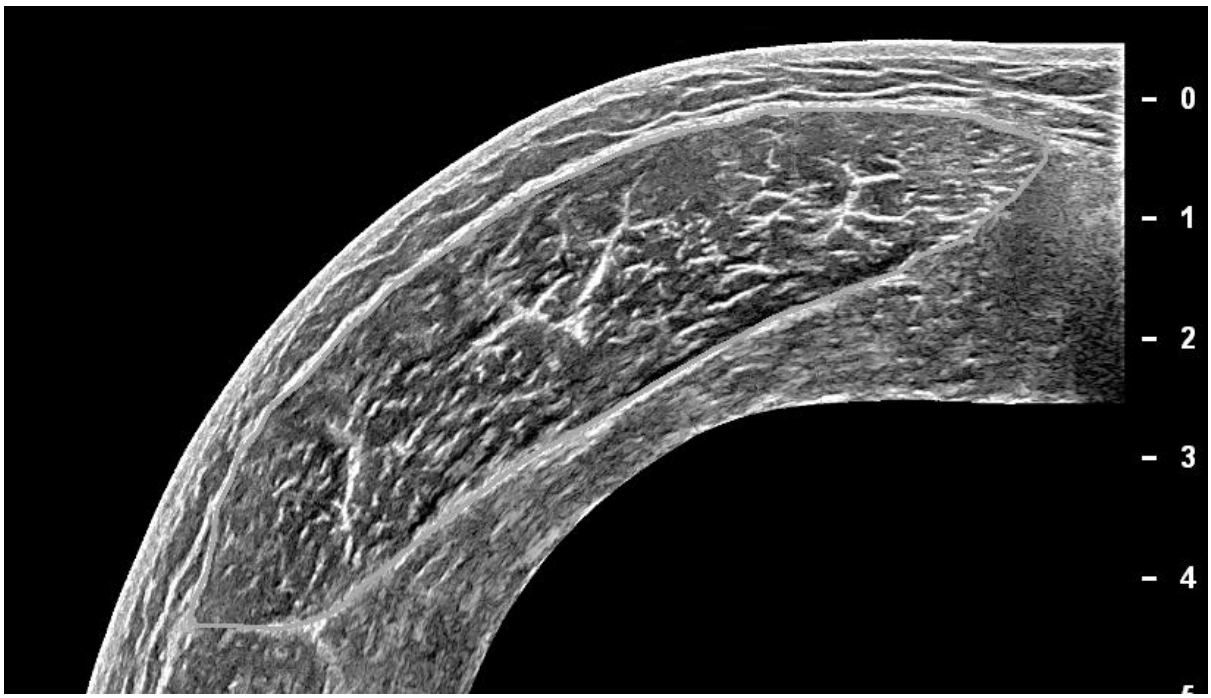
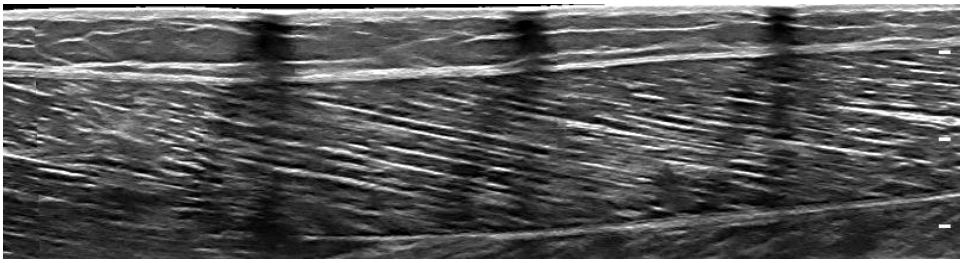


Figure 1. Representative anatomical cross-sectional area (ACSA) ultrasound EFOV image of the GM at 50% muscle length (A) and the GM ACSA outlined (B).

A



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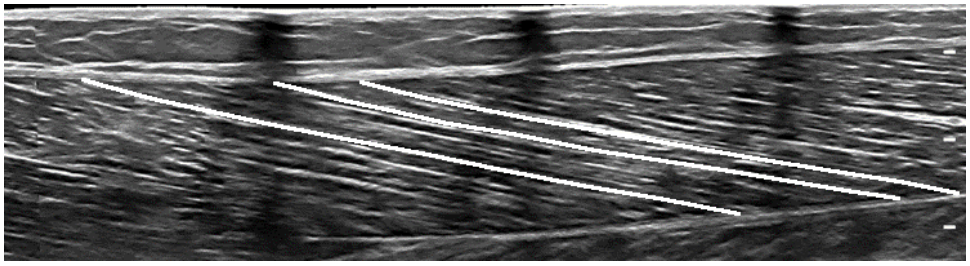


Figure 2. Representative muscle architecture ultrasound image at 50% GM muscle length. The shadows represent the reference markers placed on the skin at 60% (left), 50% (middle) and 40% (right) of the GM length (**a**). Three fascicles have been traced in the same image from lower to upper aponeurosis (**b**).

3.2.3.2 Tendon CSA and Length

Participants remained relaxed in the prone position on a therapy bed. The ultrasound probe was placed perpendicular to the skin in the sagittal plane and identified the distal MTJ of the GM and the OTJ. With the ultrasound probe placed sagittally and perpendicular to the skin, water-soluble gel aided transmission, an EFOV sagittal image was taken from the distal GM MTJ to the OTJ insertion point along the midline of the tendon (Fig. 3). Minimal but constant pressure was applied to the probe to ensure contact with the skin, while avoiding compression of soft tissue. An anthropometric tape measure was used to identify four contiguous 1 cm intervals from the AT insertion and a transverse ultrasound image was captured twice at each interval with the probe perpendicular to the skin (Fig. 4). Tendon length and CSA were manually measured using ImageJ (NIH Image, Bethesda, MD, USA).

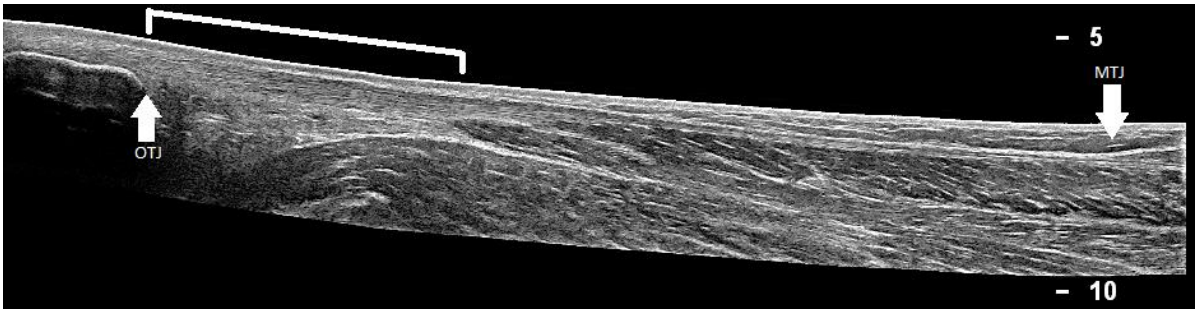
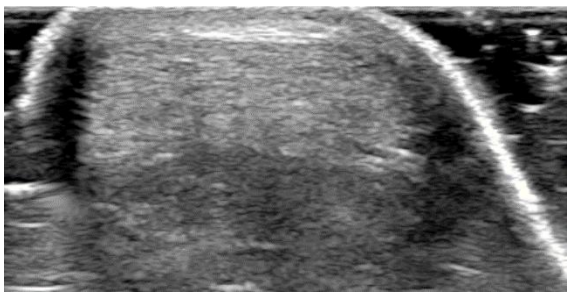


Figure 3. Representative EFOV ultrasound image of the GM tendon length from distal GM myotendinuos junction (MTJ) to osteotendinous junction (OTJ). Highlighted above is the length of the 'free' tendon from OTJ to soleus MTJ where CSA images were taken.

A



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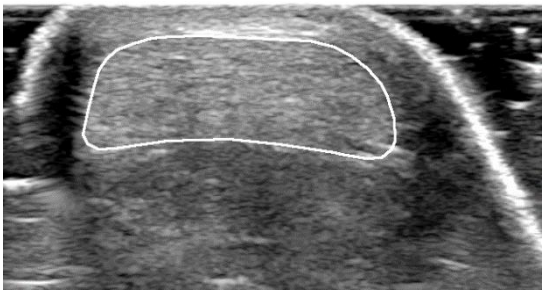

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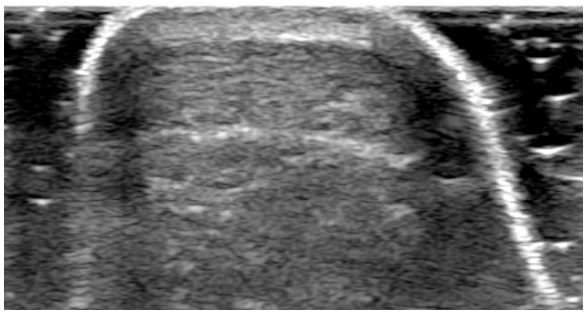

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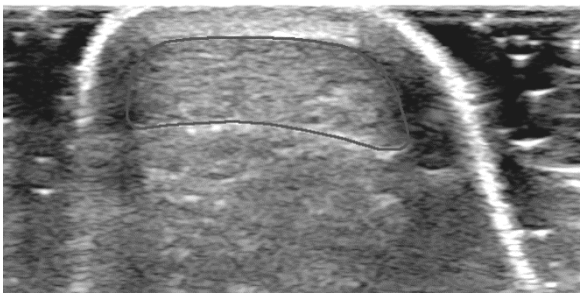

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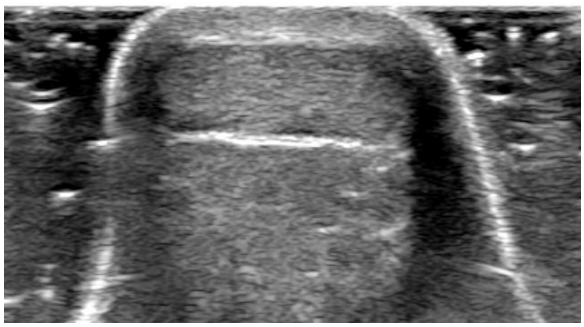

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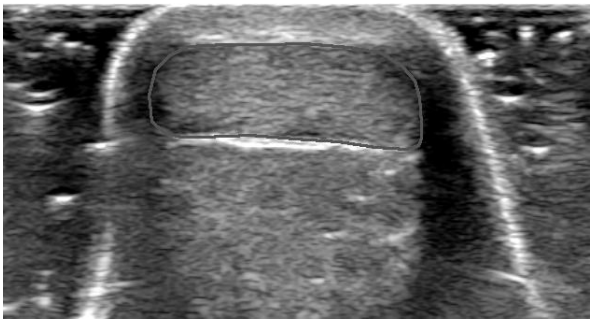


G

H

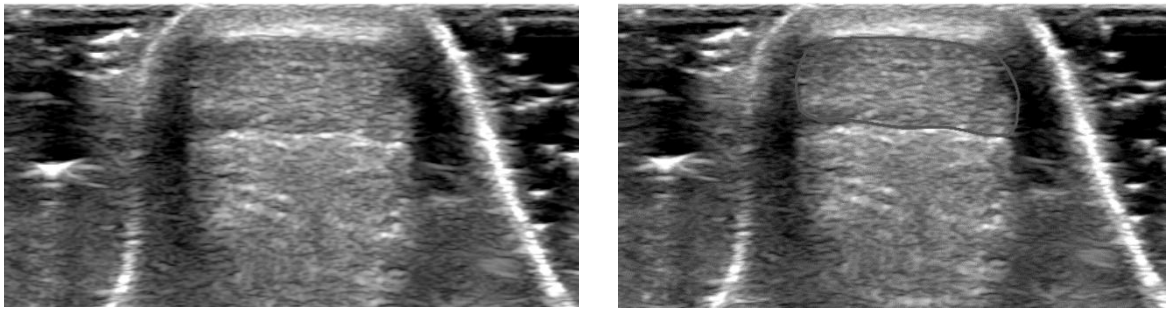


Figure 4. Representative transverse cross sectional area images of the Achilles tendon where the outline has been highlighted at 1 cm (**a** and **b**), 2 cm (**c** and **d**), 3 cm (**e** and **f**) and 4 cm (**g** and **h**) proximal to the osteotendinous junction on the calcaneus.

3.2.3.3 Maximum voluntary contraction (MVC) and GM tendon force

Participants were seated and firmly secured on an isokinetic dynamometer (Humac Norm, CSMI, Massachusetts, USA), sat in an upright position (hip angle at 85°; supine position = 180°) with their knee fully extended (0° knee joint angle) and ankle set in a neutral position (0°; 90° = full plantar flexion). The centre of rotation on the dynamometer lever arm was in line with the centre of the participants' lateral malleolus. With the foot fixed in a foot plate attachment, secured by three inextensible straps, the thigh and torso were also secured to the seat with inextensible straps, to minimise additional movement or contribution to torque production from anything other than the plantar/dorsiflexor muscles. Participants completed a standardised warm up comprising 10 isokinetic contractions at $60°\cdot s^{-1}$, gradually increasing contraction intensity. Following the warm up, participants completed three plantar flexion and three dorsiflexion isometric MVCs with the ankle set at neutral. Each trial alternated between plantar flexion and dorsiflexion, interspersed with 30 s rest and each MVC lasted for ~3 s until a plateau in the torque-time trace occurred.

Achilles tendon force (F_t) was calculated by dividing PF MVC torque (corrected for antagonist co-activation) by the Achilles tendon moment arm (d_{AT}, see below for details).

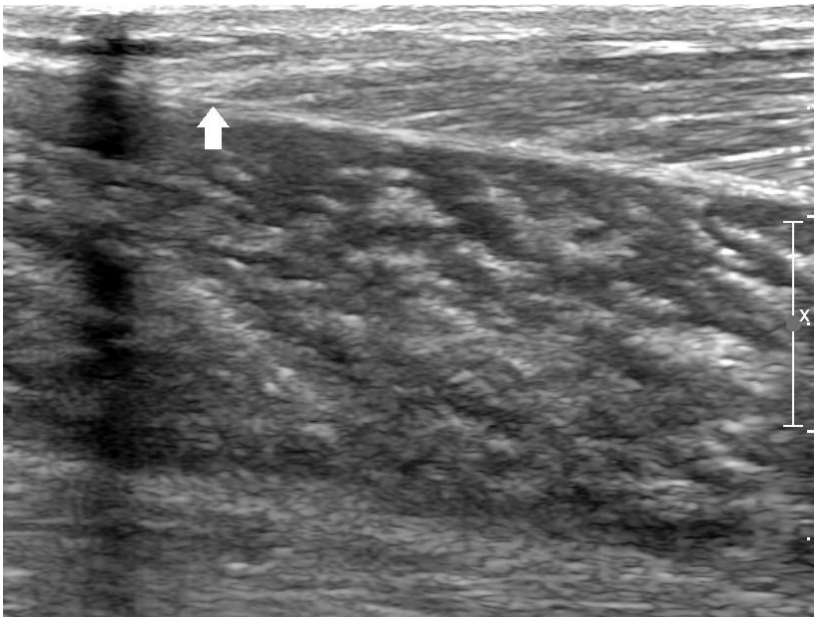
Based on the relative proportion of GM muscle volume to total PF muscle volume (Fukunaga, Roy, *et al.*, 1996), the contribution of the GM muscle to F_t (i.e. GM F_t) was assumed to be 20.3%. Therefore, GM F_t was calculated using the following equation:

$$\text{GM } F_t = (\text{PF MVC} / d_{AT}) \times 0.203$$

3.2.3.4 Tendon Elongation (Ramped MVC)

Participants were seated on the isokinetic dynamometer as per the MVC protocol and previous sub-maximal contractions and MVCs acted as pre-conditioning for the tendon (Maganaris, 2003). To assess GM tendon displacement at the MTJ, the probe was placed on the skin, sagitally along the length of the muscle/tendon (over the MTJ, which was stretched sagitally in the proximal direction). To measure the distance the MTJ travelled, a strip of 2 mm wide echo-absorbent micropore tape (3M) had to be placed on the skin transversely across the length of the tendon, approximately 1 cm proximal to the distal GM MTJ. With the micropore tape in the field of view, participants were instructed to produce a graded (ramped) contraction, progressively increasing torque output from rest over 6 s and producing MVC torque at 6 s. These images were synchronised with the torque-time and EMG-time data using an electrical square wave impulse that was visible simultaneously on the ultrasound movie file (via the ECG channel) and the AcqKnowledge (Version 5, Biopac Systems Inc., Goleta) moment/angle/EMG data collection file. Ultrasound video clips and torque-EMG traces were checked immediately after each ramped MVC, and if there were any issues (e.g. probe movement), the process was repeated. Once a satisfactory attempt was captured with the ultrasound, it was repositioned over the AT distal insertion into the calcaneus. Micropore tape was placed transversly across the AT at 2 cm proximal to the calcaneus insertion point, and the above process was repeated to assess distal GM tendon displacement.

A



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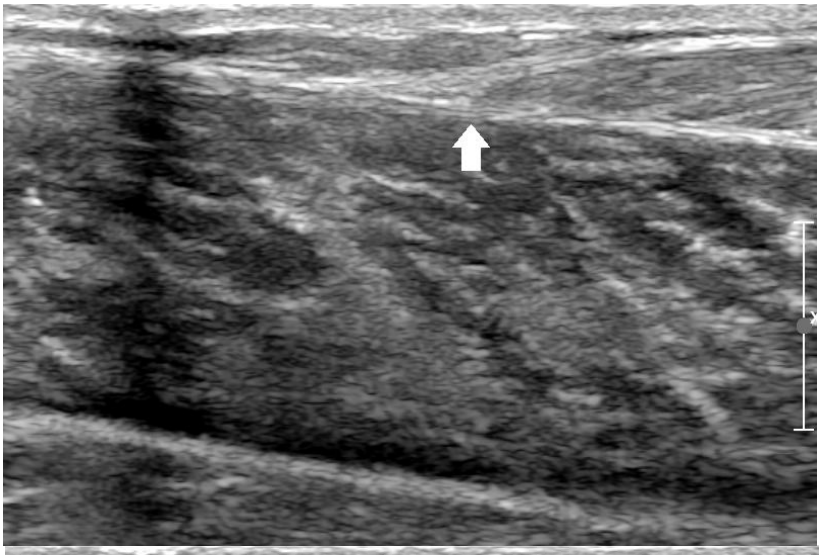

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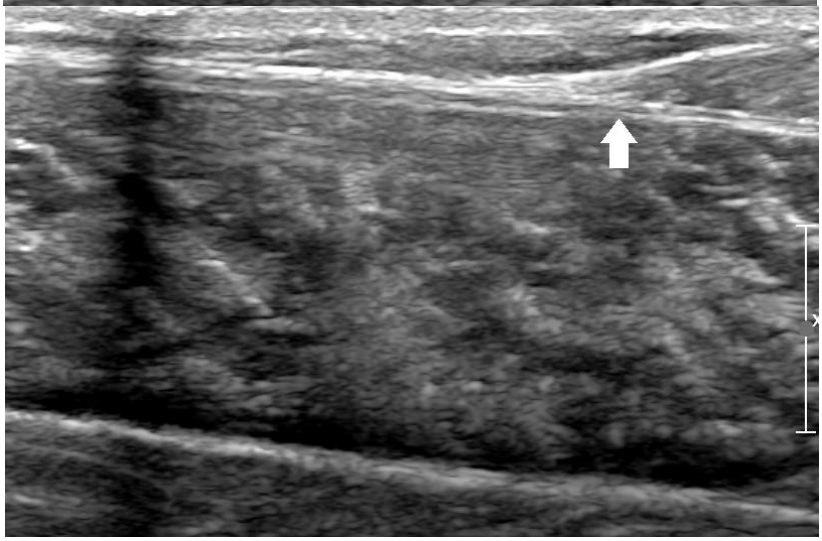
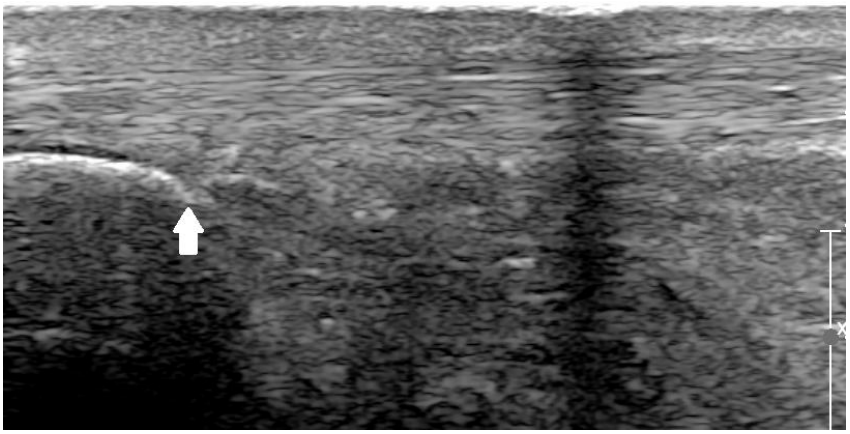


Figure 5. GM MTJ displacement during a 6 second ramped MVC at rest (**a**), 50% MVC (**b**) and 100% MVC (**c**). The shadow is the reference marker on the skin from which distance was measured to the MTJ.

A



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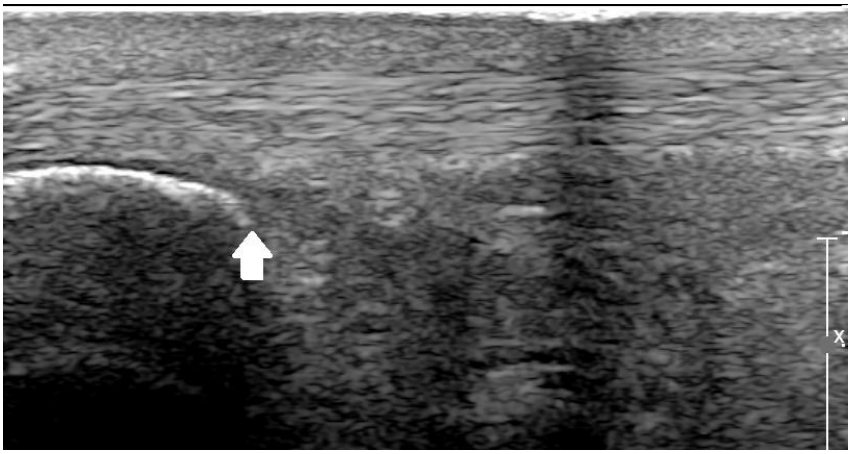

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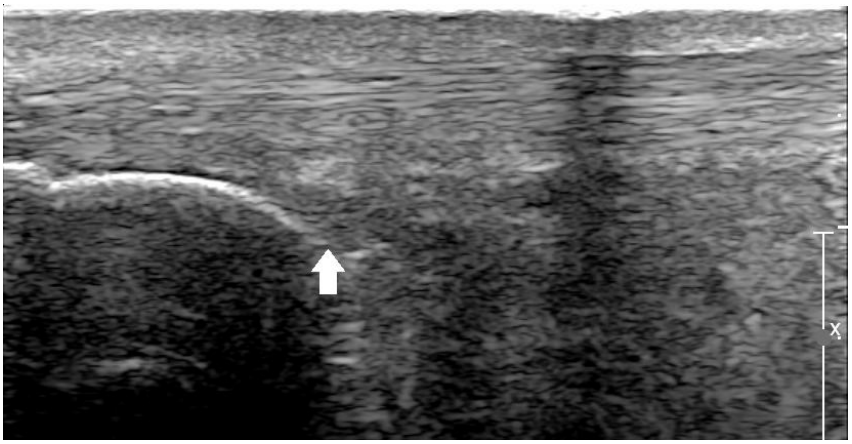


Figure 6. GM OTJ displacement during a 6 second ramped MVC at rest (**a**), 50% MVC (**b**) and 100% MVC (**c**). The shadow is the reference marker on the skin from which distance was measured to the OTJ.

3.2.3.5 Achilles tendon moment arm

Following tendon elongation and MVCs, thus ensuring the tendon was preconditioned, participants' Achilles tendon moment arm at 0° was determined using the tendon excursion method (Fath *et al.*, 2010). Participants remained seated on the IKD as per previous conditions whilst their ankle was passively rotated through +5° (plantar flexion) to -5° (dorsiflexion) at a speed of $1°{\cdot}s^{-1}$. The ankle was rotated a minimum of three times, at least twice from dorsi-flexion to plantar flexion and at least once from plantar flexion to dorsiflexion. Concurrent ultrasound video clips were recorded during these rotations, with the ultrasound probe positioned transversely over the distal MTJ of the GM where a 2 mm wide strip of echo-absorbent micropore tape was placed transversely on the skin, approximately 1 cm proximal to the distal GM MTJ as a point of reference. Participants were instructed to remain relaxed and allow the rotation to occur without the generation of plantar- or dorsiflexion torque. Ultrasound video clips and torque-time traces were checked to ensure no movement of the ultrasound probe occurred along with the absence of force production.

3.2.3.6 (Ant)agonist Muscle (Co-)Activation

Electrical activity of the GM and tibialis anterior muscles was measured during all MVCs, and passive rotations. Following appropriate skin preparation (Hermens *et al.*, 2000), two bipolar (Ag/Ag-Cl) EMG electrodes (Neuroline, Ambu, Ballerup, Denmark) were positioned 20 mm apart along the midline of the muscle, at a third of the muscle length proximally (Hermens *et al.*, 2000). An additional (reference) electrode was positioned on the lateral tibial condyle. EMG activity was sampled using a wireless transmitter (Bionomadix, 2000Hz, Biopac Systems Inc., Goleta, USA) and recorded simultaneously in AcqKnowledge along with the torque-time trace.

3.2.3.7 Countermovement Jumps

Participants performed vertical and horizontal countermovement jumps both bilaterally and unilaterally. Vertical GRF (VGRF), horizontal anterior–posterior GRF (HGRF), and medio-lateral GRF (MGRF) data were collected using an in-ground 0.9 × 0.6 m^2 force platform (9287C, Kistler InstrumentsLtd., Winterhur, Switzerland), at a sampling rate of 1000 Hz. Participants were instructed to stand still on the force platform for at least three seconds prior to initiation of the jump to provide a silent period in the force-time trace, thus allowing an accurate determination of countermovement onset (Owen *et al.*, 2014). Regarding the unilateral countermovement jumps [described in detail elsewhere (Murtagh et al., 2017)], an attempt was deemed acceptable if the femur of the contra-lateral limb did not pass behind the femur of the dominant limb in the sagittal plane of motion, as determined via visual inspection by the investigator. Participants were also required to land initially on their jumping leg before their non-jumping limb could come into contact with the floor to provide balance, which for the horizontal jumps, had to be placed in front of the jumping limb. For the horizontal countermovement jumps (HCMJ), jump distance was recorded using a tape measure running parallel to the force platform and measured to the nearest 1.0 cm, and a dowel was placed against the heel of the jumping leg upon landing, perpendicular to the tape measure (Murtagh et al., 2017, 2018a, 2018b). Jump height and projectile range were calculated for each vertical and horizontal countermovement jump respectively, and the trial with the highest or furthest jump was used in subsequent analysis.

3.2.3.8 Drop Jumps

Bilateral drop jumps were performed from a 40 cm box, where an attempt was only deemed acceptable if contact time was below 250 ms (Schmidtbleicher, 1992). Upon landing on the force platform participants were cued to jump as high as possible, whilst minimising ground contact time. Reactive strength index (RSI) was calculated by dividing jump height by

contact time, with jump height determined via flight time (Flanagan and Comyns, 2008). Vertical stiffness, the amount of centre of mass displacement experienced in response to VGRF, was calculated by dividing peak vertical force by peak centre of mass displacement during ground contact (Farley and Morgenroth, 1999; Maloney *et al.*, 2016). For the drop jumps, the attempt with the highest RSI was used for subsequent analysis.

3.2.4 Data processing

3.2.4.1 Muscle Architecture

Three fascicles were identified at 50% muscle length in the sagittal scan, all of which had to pass through the mid-point of the muscle (as identified by the micropore tape) and have a clear insertion into both superficial and deep aponeurosis to prevent any need for extrapolation or estimation of length (Franchi *et al.*, 2018). Resting fascicle length (L_f) was traced and measured from the insertion of deep to superficial aponeuroses using ImageJ software, while resting pennation angle (θ_p,) was measured from the same fascicles and taken from the angle of insertion into the deep aponeurosis (Fig. 2). Muscle thickness was also determined at the mid-point of the muscle as the distance perpendicular to the superficial aponeurosis along the shadow of the micropore tape. Anatomical cross-sectional area (ASCA) was identified using the transverse images, by manually tracing the outline of the GM at the 50% interval using ImageJ (Fig. 1). Muscle volume was calculated by adopting a truncated cone method implemented previously (Erskine *et al.*, 2017), which utilised the ACSA taken at 20, 40, 50, 60 and 80% intervals, using the below equation:

$$V_m = 1/3 \cdot d \cdot [a + \sqrt{(a.b)} + b]$$

Where d is the distance between ACSA intervals (a and b). Physiological cross-sectional area (PCSA) was then determined by dividing V_m by L_f.

3.2.4.2 MVCs

For all plantarflexion and dorsiflexion MVCs, torque was recorded using AcqKnowledge software. For the analysis of peak torque, resting baseline torque values were subtracted from the peak torque recorded across trials with the highest peak torque taken as the MVC torque for both plantarflexion and dorsiflexion. During the ramped contractions, peak torque was calculated using the same method, where 10% increments in torque were determined and added onto the resting baseline to allow for accurate identification of time occurrence. Ankle moment and angle data were recorded via an analog-to-digital converter (MP160, Biopac Systems Inc., Goleta, USA), sampled at 2000 Hz with a PC using AcqKnowledge. Torque and angle data were filtered using a low-pass at a 10-Hz edge frequency. The EMG signal was filtered using a band-pass filter set at 10 and 500 Hz. The root mean square (RMS) of the EMG signal (calculated every 200 ms) was averaged over 500 ms around (250 ms either side) peak torque for GM and TA during both PF and DF MVCs. During the ramped MVCs, the TA RMS EMG was averaged over 50 ms at every 10% PF MVC interval. Antagonist (TA) torque contribution during PF was determined and PF MVC was corrected to account for antagonist co-activation using the following equation:

(TA EMG RMS_{PF} */ Peak TA EMG* RMS_{DF}*) * DF Peak Torque) + PF Peak Torque*.

3.2.4.3 Calculation of tendon properties

Individual ultrasound movie frames were extracted at each 10% torque increment with the distance between MTJ and reference mark measured. MTJ displacement was calculated by subtracting the distance between MTJ and reference at rest from the distance at each time interval. Distal tendon displacement at the OTJ was calculated correspondingly, to quantify internal joint rotation, subtracting the distance between OTJ and reference marker at rest from the distance at each time interval (Maganaris, 2005), equating to 10% torque increments of the MVC for that trial. Following this, torque was corrected by accounting for TA co-

activation (see equation used during MVC) and then converted to force by dividing torque by the average moment arm between the two testing occasions. Force-elongation data were then plotted where individual force–elongation curves were fitted with a second-order polynomial ($R^2 > 0.95$ in all cases).

Overall tendon elongation, and the respective mechanical properties, were calculated using two different methods:

Method 1

Force-elongation data included both distal and proximal tendon displacements individually, which were each fitted with a second-order polynomial ($R^2 > 0.95$ in all cases). The polynomial function for distal displacement was subtracted from the proximal polynomial to calculate overall tendon elongation. This overall tendon displacement was fitted to a new force-elongation curve with a second-order polynomial to prevent inaccuracies through differences in maximal torque outputs between trials estimation.

Method 2

Force-elongation data included proximal tendon displacement only and was fitted with a second-order polynomial ($R^2 > 0.95$ in all cases).

Method 1 and 2

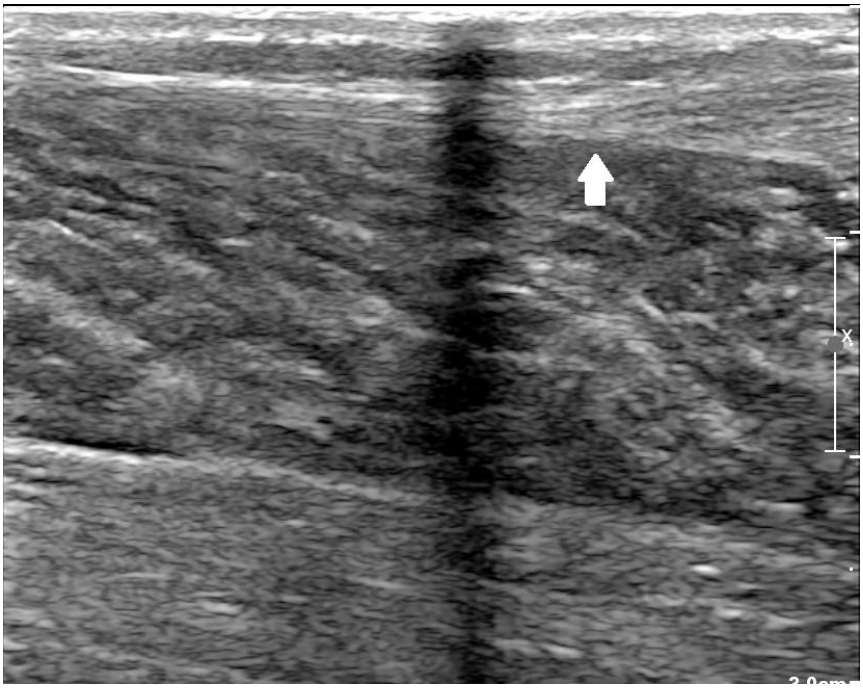
For the test-retest reproducibility analyses of tendon mechanical properties, the highest common force across each individual's two testing occasions was used per participant. When comparing Method 1 with Method 2, the highest common force between both methods was used for each individual. Mechanical stress was determined through dividing tendon force by mean tendon CSA. Tendon strain was defined as the elongation recorded during the ramped contraction (proximal minus distal displacement) divided by resting GM tendon length.

Young's modulus was calculated by dividing stress by strain. Stiffness was calculated as the change in force divided by the change in tendon elongation at 80-100% of the individual polynomial function of the ramped MVC.

3.2.4.4 Achilles tendon moment arm

Ankle joint torque and angle data were recorded in AcqKnowledge and filtered with a 10 Hz low-pass filter. Ultrasound movie files were synchronised with the ankle displacement-time and torque data, as described above, and MTJ displacement (distance between MTJ and tape) was analysed using ImageJ software, as detailed above. A linear trendline was applied to the MTJ displacement and ankle joint rotation data, which was then differentiated for 0° to provide the AT moment arm at the same ankle joint angle as the MVCs. Due to the assumption with the TE method where no internal force (through active muscular contraction or contribution through friction) are generated during passive joint rotation, equilibrium equations can be determined through the principle of virtual work (Storace & Wolf, 1979; An, Takahashi, Harrigan, & Chao, 2009;).

A



B

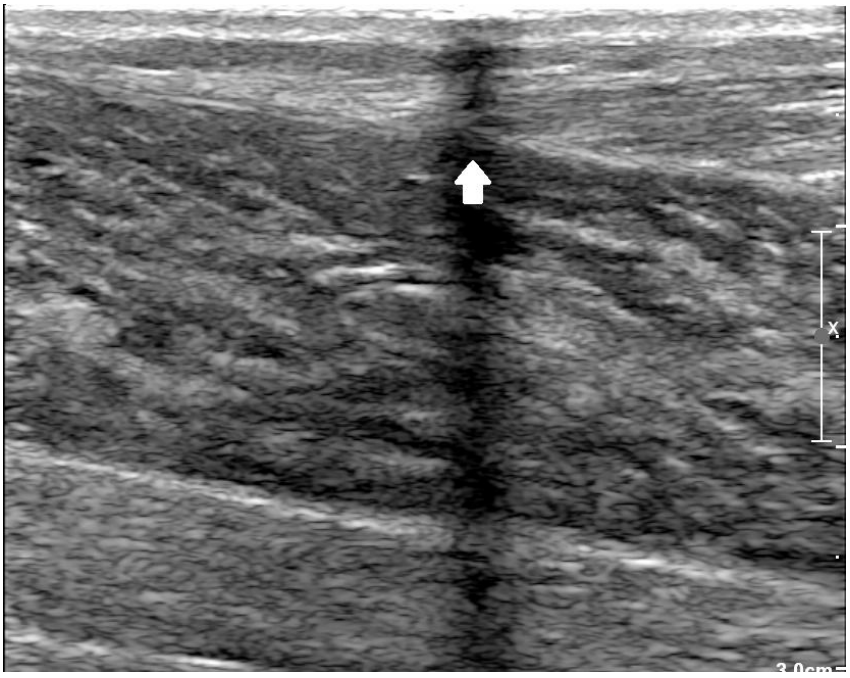


Figure 7. MTJ displacement during passive rotations at +5° (**a**) and -5° (**b**) plantar flexion.

3.2.4.5 Jump Data Analysis

Vertical and anterior-posterior (horizontal) ground reaction force (GRF) was recorded throughout the jumping protocols in AcqKnowledge. The raw force-time data were exported into a customised Excel Spreadsheet (Office 2007, Microsoft, Redmond, USA). Participant

body weight (N), taken as an average from the 'silent period' prior to the onset of countermovement, was subtracted from vertical GRF to provide a value of net vertical force. Net horizontal force was taken from the force-time trace, as it was not considered to be influenced by gravity. Centre of mass acceleration was calculated for both vertical and horizontal directions, by dividing the respective net force by participant body mass (kg). Numerical integration of both vertical and horizontal acceleration provided a calculation of vertical and horizontal velocity respectively, as per Newton's laws of motion as described elsewhere (Blazevich, 2007).

Jump height from the vertical countermovement jump trials was estimated using a calculation of constant acceleration (Dowling and Vamos, 1993):

$$Takeoff\ velocity^2 / 2*gravity$$

HCMJ distance was calculated using an equation of projectile range (Grimshaw et al., 2007):

$$(Resultant\ Take\text{-}off\ Velocity^2 * (2*Sin\ Angle\ of\ Takeoff)) / (2*Gravity)$$

Instantaneous vertical and horizontal power was measured by multiplying the respective GRF by the respective velocity for a given time point, the highest power value calculated during stance was taken as the peak power output for that vector. Only peak vertical power was recorded for the vertical countermovement jumps, whereas both peak vertical and horizontal power were measured for the horizontal countermovement jumps.

For both countermovement protocols, the onset of countermovement was identified in line with current guidelines (Owen *et al.*, 2014), as the first occurrence when the vertical force passed below five times the standard deviation of the silent period. Flight phase onset was determined as the time point when the corresponding vertical force passed below five times the standard deviation of the vertical force during flight phase. The attempt with the greatest

jump height or projectile distance was used for each participant, along with the associated variables for that attempt.

Jump height during drop jumps was calculated from an equation of flight time rather than take-off velocity, which can be influenced by the initial falling velocity (Moir, 2008):

$(0.5 * 9.81) * ((Flight\ Time / 2)^2)$

For each jump, flight time and contact time were determined using the same approach for identifying flight phase as the countermovement jumps. Both variables were used in the calculation of RSI (Flanagan and Comyns, 2008):

Flight time(s) / Contact time(s)

At the instance of ground contact, an initial velocity of -2.80m/s^{-1} was implemented, through an equation to determine instantaneous velocity of an object falling for a known height:

$\sqrt{(2 * gravity * 0.4)}$

Vertical velocity was integrated to calculate centre of mass displacement during the drop jumps and used in conjunction with vertical force to calculate vertical stiffness (Farley and Morgenroth, 1999):

Peak vertical force (N) / peak centre of mass displacement (m)

The jump with the highest RSI/jump height was used in further analysis.

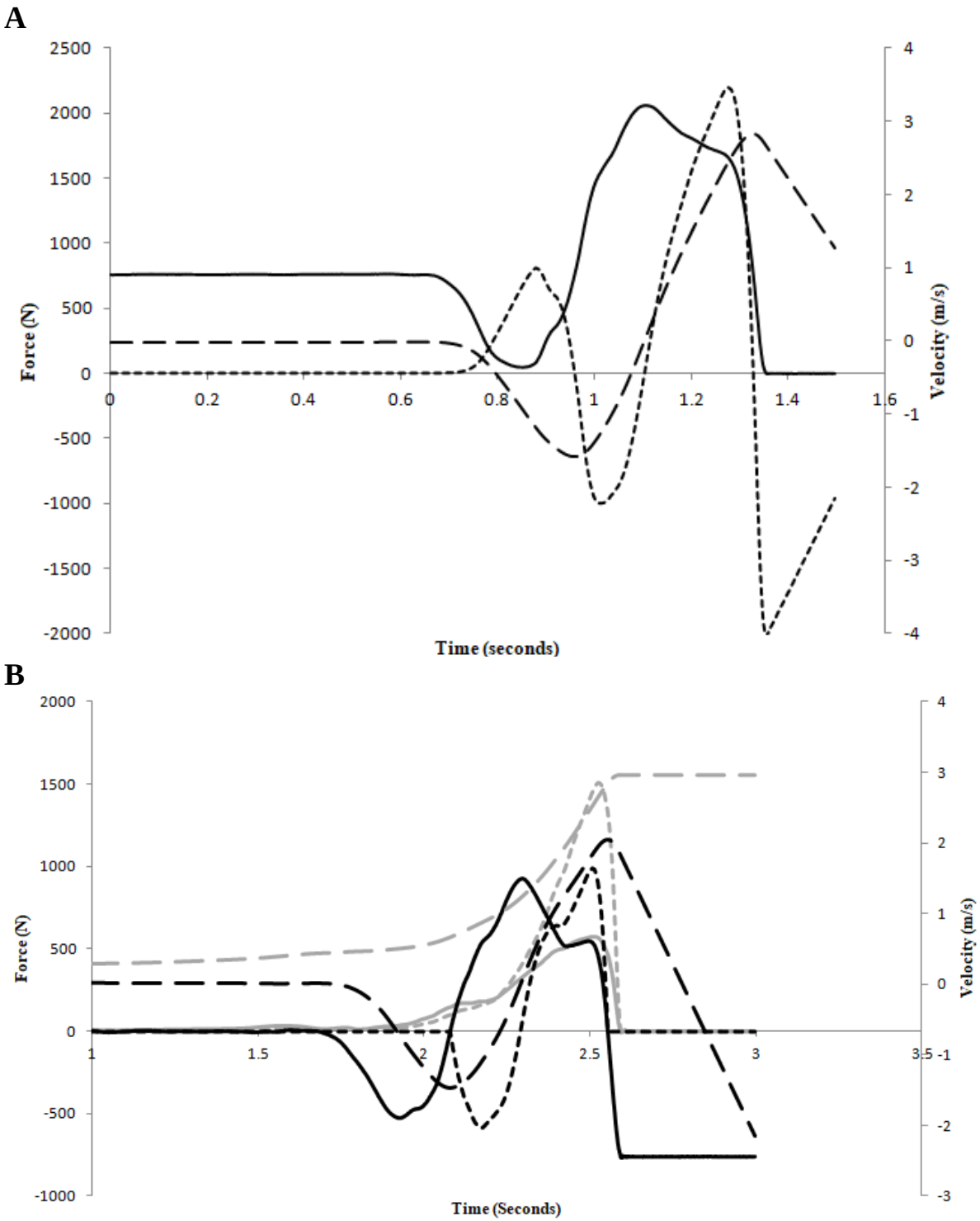


Figure 8. Force-/velocity-/power-time curve of a 77 kg participant during a: vertical countermovement jump (a); and a horizontal countermovement jump (b). Vertical ground reaction force (solid line), power (short dash); velocity (long dash) marked in black, anterior-posterior ground reaction force (solid line); power (short dash); velocity (long dash) marked in grey.

3.2.5 Statistical Analysis

Between-session reproducibility for all independent variables was assessed using a two-way random effects model (absolute agreement) intraclass correlation coefficient (ICC), as well as ratio limits of agreement (Nevill and Atkinson, 1997). Absolute reliability for all independent variables was reported as typical error (TE), while relative reliability was calculated using coefficient of variation (CV, %) (Hopkins, 2000). TE was calculated using the following equation:

Standard Deviation of Differences / (√2)

CV was determined using the following equation:

*100 * (√(Sum of squared differences / (2* sample size))/ (Average of measurement 1 and 2))*

Unless otherwise stated, data are reported as mean and standard deviation (SD) for each independent variable on each testing occasion. All statistical analyses were completed using SPSS software (Version 26, IBM SPSS Inc., Chicago, USA).

Due to loss of image quality in a small number of participants, the data from 10 participants were used in the analysis of muscle volume and PCSA, whilst the data from seven participants were used in the analyses for the between-session reliability assessment of tendon properties.

3.3 Results

3.3.1 Gastrocnemius Medialis Morphology

Good between-session reliability for all morphological characteristics of the GM muscle was demonstrated by low CVs and TEs (Table 1). Furthermore, very strong ICC values with narrow 95% confidence intervals ($p<0.001$) were found for ACSA, PCSA, MV, $L_{f,}$ MT and $\theta_{p,}$ thus demonstrating high reproducibility for these three variables. Small mean bias ratios were demonstrated for these parameters (1.00-1.03), which also signify high test-retest reproducibility (Table 1). The close ratio limits of agreement (RLoA, */÷ 1.04-1.22) indicate that 95% of the agreement ratios for each variable lay within 4 to 22% above and below the respective mean bias ratios. For example, for GM ACSA, 95% of the agreement ratios lay within 4% above and below a mean bias ratio of 1.00, i.e. between a lower limit of agreement of 1.00÷1.04 = 0.96 and an upper limit of agreement of 1.00*1.04 = 1.04. Thus, it could be stated with 95% certainty that retest GM ACSA measurements were between 4% lower and 4% higher than the first measurements.

Table 1. Test-retest reproducibility of gastrocnemius medialis (GM) muscle morphological characteristics.

Muscle characteristic	**ICC**	**95% CI**		**P Value**	**MBR (*/÷ RLoA)**	**CV (%)**	**TE (units)**
		Lower Bound	Upper Bound				
L_f	0.933	0.772	0.981	<0.001	1.01 (1.20)	6.46	0.39 (cm)
θ_p	0.903	0.667	0.972	<0.001	1.01 (1.22)	6.39	0.95 (°)
MT	0.985	0.949	0.996	<0.001	1.01 (1.06)	2.20	0.04 (cm)
GM ACSA	0.998	0.993	0.999	<0.001	1.00 (1.04)	1.19	0.17 (cm^2)

GM V_m	0.985	0.947	0.996	<0.001	1.02 (1.10)	3.37	4.92 (cm^3)
GM PCSA	0.919	0.713	0.978	<0.001	1.03 (1.19)	6.19	1.45 (cm^2)

CI, confidence interval; *MBR*, mean bias ratio; *RLoA*, ratio limits of agreement; L_f, fascicle length; θ_p, fascicle pennation angle; *MT*, muscle thickness; *ACSA*, anatomical cross sectional area; *PCSA*, physiological cross sectional area; V_m, muscle volume; *ICC*, intraclass correlation coefficient; *CV*, coefficient of variation; *TE*, typical error.

3.3.2 Gastrocnemius Tendon Morphology

Low CVs and TEs were found for the majority of Achilles tendon morphological parameters (Table 2). Whilst CSA at 1 cm displayed a higher CV and TE, only free AT length displayed unacceptably poor reproducibility. Inter-day ICC values for tendon properties were also strong for the majority of measures with narrow 95% confidence intervals. All tendon parameters, apart from the two aforementioned, showed little bias (1.00 - 1.01) and satisfactory agreement ratio limits of agreement (*/÷1.04 – 1.08). Therefore, there is a 95% chance that any that re-assessment of these characteristics would be within ± 8% of the original value. Free AT CSA measured at 1 cm and free AT length displayed poor agreement ratios (1.27 and 1.18, respectively). Achilles tendon moment arm demonstrated a moderate ICC (0.59) with wide 95% confidence intervals (-0.250 – 0.877, P=0.064), along with a poor agreement ratio (*/÷ 1.39), despite a small bias (1.08), thus suggesting low reproducibility.

Table 2. Test-retest reproducibility of Achilles tendon morphological characteristics.

Tendon characteristic	**ICC**	**95% CI**		**P Value**	**MBR (*/÷ RLoA)**	**CV (%)**	**TE (units)**
		Lower	**Upper**				
1 cm CSA	0.845	0.443	0.956	0.003	1.00 (1.27)	8.45	0.06 (cm^2)
2 cm CSA	0.856	0.491	0.959	0.002	1.01 (1.15)	4.82	0.03 (cm^2)

3 cm CSA	0.976	0.921	0.993	<0.001	1.01 (1.07)	2.41	0.01 (cm^2)
4 cm CSA	0.989	0.961	0.997	<0.001	1.00 (1.06)	2.02	0.01 (cm^2)
Mean CSA	0.974	0.91	0.992	<0.001	1.01 (1.08)	2.61	0.02 (cm^2)
GM TL	0.993	0.975	0.998	<0.001	1.00 (1.04)	1.21	0.45 (cm)
d_{AT}	0.59	-0.25	0.877	0.064	1.08 (1.39)	12.91	0.25 (cm)

CI, confidence interval; *MBR,* mean bias ratio; *RLoA,* ratio limits of agreement; *CSA,* cross sectional area; *AT,* Achilles tendon; *ICC,* intraclass correlation coefficient; *CV,* coefficient of variation; *TE,* typical error; *TL,* tendon length; d_{AT}, Achilles tendon moment arm.

3.3.3 Gastrocnemius Tendon Mechanical and Material Properties

Between-session reliability for GM tendon mechanical properties are displayed in Table 3 and descriptions of the data are provided below.

3.3.3.1 Tendon stiffness

For mechanical stiffness, the Method 1 (comprehensive method) and Method 2 (simplified method) demonstrated similar reproducibility values to one another with excellent ICC values and close 95% confidence intervals, albeit with larger than desirable CVs (>9.48%). Additionally, mean bias for both methods was low and ratio limits of agreement were poor, thus signifying the majority of data would be expected to incur a large amount of variance from the initial measurement (> */÷ 72%).

Table 3. Test-retest reproducibility of gastrocnemius medialis (GM) tendon mechanical and material properties according to Method 1 (comprehensive method) or Method 2 (simplified method).

Variable	**ICC**	**95% CI**		**P Value**	**MBR**	**CV (%)**	**TE (Units)**
(Method 1 or 2)		**Lower**	**Upper**		**(*/÷ RLoA)**		
Stiffness M1	0.959	0.790	0.993	0.001	1.04 (1.77)	13.5	117 ($N \cdot mm^{-1}$)

Stiffness M2	0.946	0.702	0.991	0.001	1.08 (1.72)	17.3	111 ($N \cdot mm^{-1}$)
Stress M1	0.993	0.960	0.999	<0.001	1.00 (1.08)	2.58	0.56 (MPa)
Stress M2	0.993	0.960	0.999	<0.001	1.00 (1.08)	2.58	0.56 (MPa)
Strain M1	0.916	0.425	0.986	0.001	1.13 (1.37)	12.3	1.08 (%)
Strain M2	0.941	0.524	0.99	<0.001	1.11 (1.27)	9.02	0.93 (%)
Elongation M1	0.897	0.269	0.983	0.002	1.13 (1.33)	12.3	1.95 (mm)
Elongation M2	0.93	0.336	0.989	<0.001	1.10 (1.22)	8.76	1.58 (mm)
YM M1	0.948	0.721	0.991	0.001	1.04 (1.80)	13.8	181 (MPa)
YM M2	0.952	0.739	0.992	0.001	1.08 (1.68)	15.0	142 (MPa)

M1, Method 1; *M2*, Method 2; *YM*, Young's modulus.

3.3.3.2 Tendon stress

Mechanical stress displayed small CVs (<2.93%) and TEs (<2.75 MPa), along with high ICCs and narrow 95% confidence intervals, which demonstrates high similarity between group means across each testing session. Furthermore, no bias was present, and limits of agreement fell within acceptable ranges outlined elsewhere (between 0.75 and 1.25) (Nevill and Atkinson, 1997), suggesting excellent reproducibility.

3.3.3.3 Tendon strain

Inter-session reliability data for strain appeared to provide consistent reliability values across both measurements. Lower CVs (9.02-12.3%) and stronger ICC values (0.916-0.941) with closer confidence intervals were found. A small bias towards the second testing occasion was present for both methods, whilst RLoA were outside the previously stated acceptable ranges (> */÷ 27%).

3.3.3.4 Tendon elongation

Reproducibility of tendon elongation displayed similar CVs (8.76-12.3%) and ICC values (0.897-0.934) to mechanical strain. However, whilst mean bias was comparable for both methods (1.10-1.13), the RLoA was slightly less than strain, incurring a minimum variance of ±22%.

3.3.3.5 Young's modulus

Similar between-session CV and TE values for both methods of determining Young's modulus were observed. Strong ICCs for both methods, coupled with narrow 95% confidence intervals, show that the agreement between values obtained on each testing occasion was high. The mean bias ratio was similar between methods, but the RLoA were consistently higher than adequate.

3.3.4 Method Comparison

Agreement between Method 1 and Method 2 for calculating mechanical and material properties of the GM tendon are displayed in Table 4 and Figure 1. Good ICC values are present for all variables (ICC = 0.800-0.895), although very wide confidence intervals were also found. Mean bias ratio shows a consistent underestimation of both stiffness and Young's modulus for method 2, whilst overestimating strain and maximal elongation. Ratio limits of agreement demonstrate a wider variance for all parameters of ± 31-60%.

Table 4. Agreement of GM tendon properties calculated using Method 2 with those using Method 1.

Variable	**ICC**	**95% CI**		**P Value**	**MBR (*/÷ RLoA)**	**CV (%)**	**TE (Units)**
		Lower	**Upper**				
Stiffness	0.876	0.211	0.963	<0.001	0.81 (1.60)	16.2	617 ($N \cdot mm^{-1}$)
Strain	0.827	-0.156	0.955	<0.001	1.23 (1.31)	15.3	0.90 (%)

Elongation	0.8	-0.18	0.948	<0.001	1.23 (1.31)	14.8	0.17 (mm)
YM	0.895	0.324	0.968	<0.001	0.81 (1.59)	16.0	0.20 (MPa)

CI, confidence interval; *MBR*, mean bias ratio; *RLoA*, ratio limits of agreement; *ICC*, intraclass correlation coefficient; *CV*, coefficient of variation; *TE*, typical error; *YM*, Young's modulus.

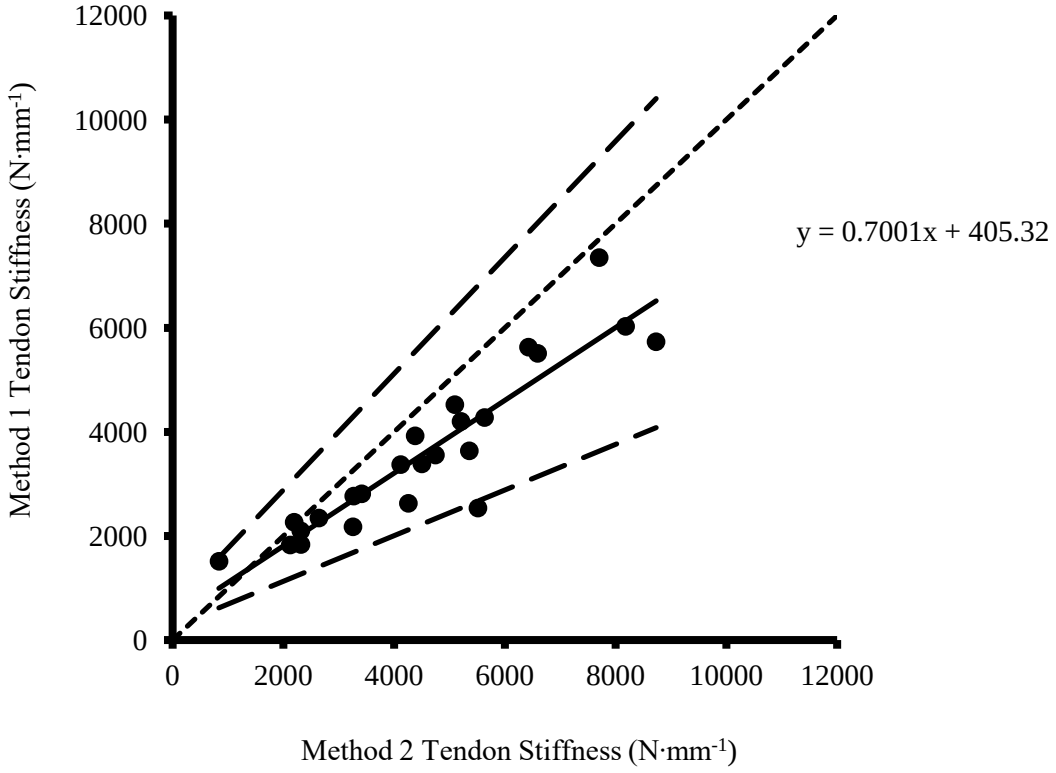


Figure 9. The bias ratio (0.812, solid line; r = 0.876; P < 0.001) and ratio limits of agreement between method 1 and method 2 (upper = 1.30, lower = 0.51, dashed lines) used to calculate absolute stiffness of the gastrocnemius tendon; dotted line = line of identity; *n* = 24.

3.3.5 Jump Variables

Between-session reproducibility of bilateral vertical countermovement jump (CMJ) variables are presented in Table 5. Coefficient of variance analysis revealed good to low values for all parameters (4.23-9.94%). Weak-to-moderate intra-class coefficient correlations with wide confidence intervals were found for all variables, where only peak vertical power achieved statistical significance (P = 0.021). Small mean bias and satisfactory RLoA were present for

jump height and take-off velocity, whilst peak power demonstrated larger than acceptable mean bias and large RLoA (±39%).

Table 5. Test-retest reproducibility of bilateral vertical countermovement jump parameters

Variable	ICC	95% CI		P Value	MBR	CV (%)	TE (units)
		Lower	Upper		(*/÷ RLoA)		
Take-Off Velocity	0.391	-1.358	0.830	0.224	0.99 (1.14)	4.44	0.13 m/s
Peak Vertical Power	0.792	0.305	0.942	0.008	0.96 (1.39)	9.94	23.08 W/kg
Jump Height	0.402	-1.281	0.832	0.215	0.98 (1.29)	8.86	3.70 cm

CI, confidence interval; *MBR,* mean bias ratio; *RLoA,* ratio limits of agreement; *ICC,* intraclass correlation coefficient; *CV,* coefficient of variation; *TE,* typical error.

Unilateral vertical CMJ test-retest reliability outputs are displayed in Table 6. Strong ICC values, with narrow 95% confidence intervals and low P values were found for take-off velocity and jump height, demonstrating a very good association between testing occasions. However, the ICC value for peak vertical power was moderate (0.579), with very wide confidence intervals (-.0574, 0.881). Slightly lower CVs were found for jump height and take-off velocity during unilateral vertical countermovement jumps than for bilateral, but vertical power produced a large reproducibility value (16.8%). Mean bias was similar across all variables and within a suitable range (0.99-1.05), however, RLoA for peak vertical power was again higher than required for appropriate reproducibility (±55%).

Table 6. Test-retest reproducibility of unilateral vertical countermovement jump parameters

Variable	ICC	95% CI		P Value	MBR	CV (%)	TE (units)
		Lower	Upper		(*/÷ RLoA)		

Take-Off Velocity	0.941	0.804	0.983	<0.001	1.02 (1.13)	4.33	0.09 m/s
Peak Vertical Power	0.579	-0.574	0.881	0.094	0.99 (1.55)	16.8	19.57 W/kg
Jump Height	0.96	0.868	0.988	<0.001	1.05 (1.29)	8.16	1.87 cm

CI, confidence interval; *MBR*, mean bias ratio; *RLoA*, ratio limits of agreement; *ICC*, intraclass correlation coefficient; *CV*, coefficient of variation; *TE*, typical error.

Test-retest reliability of bilateral horizontal CMJ variables demonstrated strong values consistently for horizontal peak power, projectile range, resultant take-off velocity and measured distance (Table 7). These variables possessed low CVs (2.77-8.04), good ICCs (0.732-0.932), although the confidence intervals were wider than desirable for all but measured distanced. The mean bias for these parameters was excellent (0.98-1.02) and acceptable RLoA demonstrated that the amount of variation which could be explained by the error within the test was ±9-26%. In contrast, vertical peak power repeatedly provided poor reproducibility values through CV (33.1%), ICC (0.321, 95% CI -1.127 – 0.801, P=0.254) and RLoA (1.89) demonstrating large test-retest variation associated with this measurement.

Table 7. Test-retest reproducibility of bilateral horizontal countermovement jump parameters

Variable	**ICC**	**95% CI**		**P Value**	**MBR**	**CV (%)**	**TE**
		Lower	**Upper**		**(*/÷ RLoA)**		**(units)**
Vertical Peak Power	0.327	-1.127	0.801	0.254	1.11 (1.89)	33.1	35.64 Watts/kg
Horizontal Peak Power	0.732	0.058	0.923	0.022	0.98 (1.26)	8.04	13.93 Watts/kg
Projectile Range	0.843	0.460	0.955	0.003	1.02 (1.20)	6.99	9.34 cm

Resultant Take-Off Velocity	0.857	0.51	0.959	0.002	1.01 (1.09)	3.22	0.12 m/s
Measured Distance	0.932	0.77	0.980	<0.001	0.99 (1.09)	2.77	5.27 cm

CI, confidence interval; *MBR*, mean bias ratio; *RLoA*, ratio limits of agreement; *ICC*, intraclass correlation coefficient; *CV*, coefficient of variation; *TE*, typical error.

Reproducibility of unilateral horizontal CMJ variables followed a similar pattern to the bilateral equivalent outlined above. Vertical peak power provided poor values of reliability, whilst measured distance was consistently the most reliable and provided the lowest CV. ICCs for the remaining variables were moderate-to-good, but had very wide confidence intervals and were non-significant (P>0.05). Whilst little mean bias was observed for horizontal peak power and projectile range (0.98 and 0.99 respectively), the RLoA demonstrated that repeated measurements could lie within ±31-37% of the original measurement. Measured distance and resultant take-off velocity were the only two variables to possess little mean bias (0.99) and demonstrate acceptable RLoA (±16-17%).

Table 8. Test-retest reproducibility of unilateral horizontal countermovement jump parameters

Variable	**ICC**	**95% CI**		**P Value**	**MBR**	**CV (%)**	**TE**
		Lower	**Upper**		**(*/÷ RLoA)**		**(units)**
Vertical Peak Power	0.59	-0.430	0.882	0.081	1.08 (1.84)	26.0	16.5 Watts/kg
Horizontal Peak Power	0.758	0.145	0.931	0.016	0.98 (1.31)	9.49	99.5 Watts/kg
Projectile Range	0.574	-0.631	0.880	0.099	0.99 (1.37)	11.0	10.2 cm
Resultant Take-Off Velocity	0.606	-0.489	0.889	0.080	0.99 (1.16)	5.22	0.16 m/s

Measured Distance	0.952	0.839	0.986	<0.001	0.99 (1.17)	4.69	6.54 cm

CI, confidence interval; *MBR*, mean bias ratio; *RLoA*, ratio limits of agreement; *ICC*, intraclass correlation coefficient; *CV*, coefficient of variation; *TE*, typical error.

Inter-session reproducibility values for vertical drop jumps are exhibited in Table 9. Jump height, flight time and contact time produced the lowest CVs amongst all variables (5.35-9.91%), whilst reactive strength index, vertical stiffness and peak force demonstrated higher CVs (10.2-21.5%). However, only vertical stiffness and peak force displayed strong ICC with narrow confidence intervals and significant a P value (P<0.001). In contrast, vertical stiffness exhibited the greater RLoA (± 65%) of all the parameters, despite very similar mean bias (1.01).

Table 9. Test-retest reproducibility of bilateral vertical drop jump parameters

Variable	**ICC**	**95% CI**		**P Value**	**MBR**	**CV (%)**	**TE (units)**
		Lower	**Upper**		**(*/÷ RLoA)**		
Jump Height	0.632	-0.320	0.895	0.062	1.01 (1.40)	9.91	3.38 cm
Contact Time	0.658	-0.287	0.904	0.053	1.00 (1.25)	7.98	0.02 s
Flight Time	0.566	-0.658	0.878	0.103	1.01 (1.18)	5.35	0.03 s
Reactive Strength Index	0.628	-0.361	0.894	0.066	1.01 (1.39)	10.2	0.27 AU
Peak Force	0.926	0.740	0.979	<0.001	1.03 (1.47)	21.5	539 N
Vertical Stiffness	0.945	0.814	0.984	<0.001	1.01 (1.65)	18.7	85.71 ($N \cdot m \cdot kg^{-1}$)

CI, confidence interval; *MBR*, mean bias ratio; *RLoA*, ratio limits of agreement; *ICC*, intraclass correlation coefficient; *CV*, coefficient of variation; *TE*, typical error.

3.4 Discussion

The aims of this study were to describe the methodologies commonly used in this thesis in detail, to investigate the reproducibility of frequently used methods for assessing GM MTU characteristics and jump assessments used to monitor performance in elite athletes, and to compare a comprehensive (Method 1) and simpler (Method 2) method for calculating GM tendon mechanical and material properties. The main findings were that morphology of the GM and GM tendon can be measured with excellent reproducibility. Mechanical properties of the GM tendon can be calculated with varying degrees of reliability; however, this is not dependant on the method used, as both approaches demonstrated comparable reliability, and the simpler method was in strong agreement with the more comprehensive method regarding the majority of tendon variables. Vertical unilateral jump parameters were slightly more reproducible than the bilateral equivalent, whilst bilateral horizontal jumps provided more reliable outputs than their unilateral counterparts. Peak vertical power proved to be more reliable in VCMJ when compared with horizontal jumps. Furthermore, stronger reproducibility of both vertical and horizontal power occurred during bilateral, rather than unilateral, jumps. Additionally, temporal parameters were more reliable than kinetic outputs during drop jumps. These results can inform researchers and practitioners alike as to which assessments are sensitive enough to detect meaningful differences (e.g. between populations) and/or changes over time (e.g. determining the efficacy of a training programme).

The reproducibility of GM fascicle length assessment was excellent (ICC = 0.933), with only small variation between days (CV = 6.26%). These values are in line with those reported previously, however this is the first study to utilise EFOV imaging technology to do so, instead of using larger probe widths or extrapolation techniques (Mohagheghi *et al.*, 2007; Legerlotz, Smith and Hing, 2010; Raj, Bird and Shield, 2012; McMahon, Turner and Comfort, 2016). Our findings are in line with data collected in other muscle groups also

utilising EFOV imaging (Noorkoiv *et al.*, 2010; Nelson, Dewald and Murray, 2016). For muscle thickness and fascicle pennation angle, very similar reproducibility values were found by McMahon, Turner and Comfort, (2016), potentially due to the similar population investigated and methodology. Our muscle size data (MT, ACSA, PCSA and V_m) displayed some of the highest reproducibility values with very low CVs (1.24-6.19%) and excellent ICCs (0.919-0.998), both of which are slightly better than those reported previously for EFOV imaging of the GM (Scott *et al.*, 2012; Rosenberg *et al.*, 2014). This is the first study to determine the between-session RLoA for GM morphology, where for all characteristics, a very low mean bias was observed between sessions (1.00-1.03) along with small ratio limits of agreement (1.04-1.22). Therefore, researchers using the same approach employed in the current study can be confident that the test-retest variability of GM architecture parameters ranges from 4 to 22%.

Similarly, ultrasound imaging of the distance between GM MTJ and the AT insertion displayed excellent reproducibility in line with previous reports using EFOV imaging (Ryan *et al.*, 2013; Weisskirchner *et al.*, 2015; Silbernagel *et al.*, 2016; Brouwer *et al.*, 2018). Interestingly, AT CSA demonstrated superior reproducibility at proximal locations (3-4cm) across all reliability statistics. A similar pattern was observed by Intziegianni *et al.*, (2015), who also reported greater reliability of CSA 4-6 cm proximal to the calcaneal insertion. Difficulty imaging distal AT regions could stem from the proximity to the calcaneal bone and naturally wider border of the tendon at that location, which may impair image quality and cause errors when manually tracing its outline. In contrast to the findings of Intziegianni *et al.*, (2015), taking an average of the CSA across the four sites along the tendon in the current investigation provided similar reliability to proximal locations, which coincided with absolute stress of the AT displaying highly reproducible values. Given the heterogeneous nature of CSA along the length of the AT and the regional hypertrophy which can occur with training

(Magnusson and Kjaer, 2003; Holzer *et al.*, 2018), calculating a mean CSA may circumvent issues arising from taking a singular CSA image.

Further to the morphology of the tendon, estimating the AT moment arm using the tendon excursion method provided poor between-session reliability. Very wide 95% confidence intervals (-0.25 – 0.877) and large RLoA (1.39) would suggest large between test variance is associated with this method, which is supported by high CV (12.9%), and is similar to the 11.7% test-retest CV reported recently (Buffey *et al.*, 2020) but slightly higher than the 4.9-9.7% CVs reported by (Fath *et al.*, 2010). The higher test-retest reproducibility for our AT moment arm assessment could be attributed to the limited (±5°) range of motion during passive rotations. For example, previous investigations have rotated the ankle through a greater range, which was not achievable in the present study due to severe discomfort during dorsiflexion, but may be negated through a prone or supine position (Fath *et al.*, 2010, 2013; Fletcher and Macintosh, 2018). Using a larger range of motion would allow for the collection of more data points, which could permit a smoothing of data for an improved estimate of moment arm.

Large discrepancies with moment arm measurement required using an average value from the two testing sessions when calculating force-elongation properties. Maximal elongation and peak strain demonstrated mostly satisfactory reproducibility (e.g. low CVs, TEs, and high ICCs), however, large RLoA suggested that, despite a strong relationship between the two occasions, the agreement of the two trials was poor. Similarly, tendon stiffness and Young's modulus portrayed conflicting reliability results. Comparison across two methods for calculating mechanical properties of the GM tendon demonstrated inconsistent findings. Comparable ICC values were present between methods, whilst CVs were lower for the comprehensive method incorporating proximal *and* distal displacement of tendon. However,

RLoA for both methods were poor. Over estimation of tendon elongation occurred in absence of distal displacement measurement, which is in line with previous observations (Magnusson *et al.*, 2001; Muramatsu, Muraoka, Takeshita, Kawakami, Hirano, Fukunaga, *et al.*, 2001; Arampatzis *et al.*, 2005; Karamanidis *et al.*, 2005; Maganaris, 2005; De Monte *et al.*, 2006; Kongsgaard *et al.*, 2011). However, figure 1 displays a linear regression equation, which can be used as a correction factor when only quantifying proximal tendon displacement as a measure of mechanical properties. Therefore, the use of an alternative time-efficient technique can be implemented with confidence, due to the comparable reliability values and linear association to a more comprehensive method.

Inconsistent approaches to calculating GM tendon properties limit comparison with previous investigations, which demonstrate wide-ranging reliability values (Kubo, Kanehisa and Fukunaga, 2001, 2005; Muramatsu, Muraoka, Takeshita, Kawakami, Hirano and Fukunaga, 2001; Kubo *et al.*, 2002; Mahieu *et al.*, 2004; Fouré, Nordez and Cornu, 2010; Keitaro Kubo *et al.*, 2014). Several factors could explain reproducibility discrepancies between the present findings and those previously reported, including the force range measured; positioning of participants on the isokinetic dynamometer (e.g. seated *vs.* prone/supine); and identification of the resting tendon length. In addition, one prominent aspect to consider is the quantification of distal tendon displacement. Several approaches have been utilised within the literature, including the use of a potentiometer to directly measure change in heel position, or an electrical goniometer to measure joint angle during contraction (Magnusson *et al.*, 2001; Stenroth *et al.*, 2016). The current investigation did not implement either of these approaches, which may limit comparison with other studies utilising different methods. However, measurement of the OTJ displacement would directly determine the internal joint rotation of the calcaneus, which, when used in conjunction with echo-absorbent skin markers, can be used to determine overall GM tendon length change.

A third focal point of this study was the reproducibility of kinetic and temporal parameters measured during a variety of jumping tasks. Height attained during a bilateral VCMJ is frequently used as a performance outcome, however, the reproducibility of which, determined via take-off velocity, was worse than previously reported (McMahon *et al.*, 2018). Slightly larger CVs in the current study could be due to different calculations used, but a generally poor association between outputs (ICC = 0.463, 95% confidence intervals -0.906, 0.846) and poor RLoA (1.27) suggest it is a highly variable parameter. In comparison, the unilateral equivalent demonstrated better reproducibility across all parameters, which provided very similar CV and ICC values reported previously for unilateral take-off velocity and jump height (Meylan *et al.*, 2012).

Conversely, utilising a tape measure to manually measure HCMJ distance, we have demonstrated excellent reproducibility during both a bilateral and unilateral CMJ, which is in line with previous reports (Meylan *et al.*, 2009, 2010). Projectile range displayed similar CVs and ICCs for bilateral HCMJ to those reported by Meylan *et al.*, (2012) (CVs = 3.8-6.0, ICCs = 0.87-0.96), although RLoA and CV were poor for unilateral HCMJ, the reliability of which has not been investigated previously. Limited data exist to compare with our bilateral HCMJ results, as unilateral HCMJs have received more attention within the literature. Further to jump distance, kinetic parameters displayed variable reproducibility. Low CVs, minimal mean bias and low RLoA were present for resultant take-off velocity compared with peak horizontal power, although both variables did appear more reliable during bilateral jumps. Reliability values for peak horizontal power were similar to those reported elsewhere (Meylan *et al.*, 2012).

Interestingly, whilst horizontal power displayed good reliability across both HCMJs, vertical power displayed mixed reliability outputs. Measured during horizontal jumps, peak vertical power displayed large CVs, very wide 95% confidence intervals and large RLoA. However,

during bilateral vertical CMJs, peak power was in line with CVs previously reported for unilateral vertical CMJs (Cormack *et al.*, 2008; Meylan *et al.*, 2009, 2012) (4.2-9.1%). Furthermore, peak power measured during unilateral jumps produced larger CVs than reported elsewhere, potentially a result of different jumping technique employed (Meylan *et al.*, 2009, 2012).

Alongside CMJ, bilateral drop jump variables provide an insight into alternative physical qualities. Temporal parameters displayed the strongest values of reproducibility, although RSI CV was higher than previously reported (Feldmann *et al.*, 2012; Byrne *et al.*, 2017; Maloney, Richards and Fletcher, 2018). This could be caused by slightly greater variance in contact times, which may be the result of altered technique between sessions, possibly requiring an additional familiarisation occasion. Vertical stiffness however, displayed a very similar CV and ICC to previous reports using comparable methods (Maloney, Richards and Fletcher, 2018).

To conclude, EFOV ultrasound techniques can be implemented with confidence to determine morphological characteristics of the GM muscle and tendon. Researchers should carefully consider the ankle range of motion when utilising the TE method for determining AT moment arm. Mechanical properties of the GM tendon can be calculated reliably, whereby a simpler method to measure GM tendon mechanical and material properties was found to agree strongly with a more comprehensive method, thus providing a valid alternative when time and resources are limited (e.g. when assessing elite athletes). Jump performance outputs can be considered more reliable when using unilateral VCMJ and bilateral modalities for HCMJ. Caution should be used when interpreting changes in performance outputs from drop jumps due to the slightly poorer reliability compared to CMJs.

Chapter Four

Gastrocnemius medialis muscle properties and their relationship with jump performance in elite youth soccer players and control participants

Prelude

The work in chapter 4 utilises the methods described in the previous chapter to provide an insight into the specific morphological properties of the GM in a group of elite male youth soccer players and how their physiological characteristics can help influence physical performance. This chapter will seek to highlight any characteristics that may be specific to the group of soccer players, by comparing them to a cohort of recreationally active young men, whilst also identifying relationships with key markers of physical performance also outlined in chapter 3. It is hoped that this information will identify specific physiological characteristics that are beneficial for physical performance in elite youth soccer players.

Abstract

The aim of this study was to (i) investigate gastrocnemius medialis (GM) muscle properties in elite youth soccer players (ESP) and recreationally active control participants (CON); and (ii) determine the relationship between GM morphological characteristics and jump performance parameters. Twenty-two ESP (age=18.8 ± 1.4 years, height=1.82 ± 0.08 m, weight = 75.1 ± 5.9 kg; mean ± SD) and 12 healthy male CON (age=22.2 ± 2.9 years, height=1.75 ± 0.05 m, weight=71.6 ± 7.4 kg; mean ± SD) participated in this study. GM morphology and architecture was measured using ultrasonography at 50% GM muscle length, isokinetic dynamometry was used to determine isometric plantar flexion and dorsiflexion strength. Participants performed a series of unilateral and bilateral countermovement jumps (CMJ) and bilateral drop jumps on a force platform. ESP (17.4 ± 2.5°) had a larger GM fascicle pennation angle (θ_p) compared to CON (14.3 ± 1.2°, P<0.001), which correlated with unilateral vertical CMJ height and vertical power (r=0.449 and 0.432, P<0.05), whereas fascicle length correlated inversely with the same jump variables (r=-0.417 and -0.529, P<0.05, respectively). During bilateral horizontal CMJ, GM anatomical cross-sectional area and thickness correlated with peak horizontal power (r=0.407 and 0.362, $p \leq 0.038$). However, GM muscle volume correlated inversely with jump distance and vertical power during bilateral vertical and horizontal CMJ (r=-0.840 to -0.633, $p \leq 0.05$). These results suggest that a greater number of GM sarcomeres arranged in parallel, not series, could be beneficial for unilateral jump performance.

4.1 Introduction

Physical profiling of elite athletes is often used to identify key performance indicators (KPI) of the sport. Acceleration and maximal sprinting are known KPIs in competitive soccer (Faude, Koch and Meyer, 2012; Emmonds *et al.*, 2016), and are underpinned by power and reactive strength qualities (Morin *et al.*, 2012; Colyer *et al.*, 2018; Douglas *et al.*, 2020). Countermovement jumps (CMJ) and drop jumps have been implemented to investigate the determinants of power (Murtagh et al., 2017) and reactive strength performance (Maloney, Richards and Fletcher, 2018; Douglas *et al.*, 2020).

Previous studies have sought to identify the neuromuscular characteristics that underpin performance in jumping tasks. One of the most consistent findings has been the relationship between muscle size (thickness and physiological cross-sectional area) of the vastus lateralis (VL) (Mangine *et al.*, 2014; Secomb, Lundgren, *et al.*, 2015; Murtagh, Nulty, *et al.*, 2018) and lateral gastrocnemius (LG) (Earp *et al.*, 2010; Secomb, Nimphius, *et al.*, 2015) with jump variables (height, power, peak force) during a squat, countermovement, and drop jump. Further positive relationships have been observed between VL and LG fascicle pennation angle (the angle at which the muscle fascicles insert into the lower aponeurosis, thought to be determined by muscle fibre cross-sectional area (Aagaard *et al.*, 2001; Degens, Erskine and Morse, 2009) with jump height, mean power, peak power and peak velocity in vertical jumps (Alegre *et al.*, 2005; Earp *et al.*, 2010; Secomb, Lundgren, *et al.*, 2015; Secomb, Nimphius, *et al.*, 2015; Murtagh, Nulty, *et al.*, 2018). Interestingly, the opposite relationship with fascicle pennation angle was found for horizontally oriented jumps (Murtagh, Nulty, *et al.*, 2018), thus suggesting a direction-specific relationship in connection to vertical force application variables. This could suggest that muscle architecture is only a determinant of the vertical aspect of force and power production, even during jumps in a non-vertical direction.

However, inconsistent reports have been published following training interventions where increases in muscle size have occurred without changes to jump height (Fouré, Nordez and Cornu, 2010), whilst others have shown jump height to improve in the absence to changes in muscle size (Blazevich *et al.*, 2003). Rather than contradicting previous reports, these findings could highlight the importance of determining power output during a jump rather than simply height attained. This could be of particular importance given that power outputs can help differentiate athletic status when jump height cannot (Murtagh *et al.*, 2017). Similarly, leg stiffness and reactive strength index (RSI) could also be considered the KPIs during a drop jump rather than jump height. However, limited research exists exploring the potential influence muscle architecture has on these parameters, favouring instead to report relationships only with jump height (Earp *et al.*, 2010, 2011).

Furthermore, there is a distinct lack of research identifying the important characteristics of the gastrocnemius medialis (GM) for jumping performance (Dobbs, Gill, *et al.*, 2015; Pentidis *et al.*, 2020). Findings from studies investigating the VL cannot be generalised to all muscles due to the different behaviour of fascicles and tendinous structures during jumping tasks (Ishikawa, Niemelä and Komi, 2005; Sousa *et al.*, 2007). Therefore, given the importance of the plantar flexors and Achilles tendon in jumping and running based tasks (Bobbert, Huijing and van Ingen Schenau, 1986b; Hamner, Seth and Delp, 2010; Dorn, Schache and Pandy, 2012; Hamner and Delp, 2013; Maloney *et al.*, 2016; Kipp *et al.*, 2018; Schache *et al.*, 2019), further research is necessary to understand how specific characteristics of this muscle can influence performance in jumping actions pertinent to soccer.

The aims of this study were therefore to (i) investigate GM muscle strength and morphology in elite youth soccer players and recreationally active control participants; and (ii) explore the relationship between GM morphological characteristics and performance outcomes in a series of jumping tasks. It was hypothesised that muscle size and fascicle pennation angle would

correlate positively with peak power during countermovement jumps, whilst plantar flexion force was hypothesised to correlate with leg stiffness.

4.2 Method

4.2.1 Participants

Thirty-four healthy young men, comprising 22 elite youth soccer players (ESP; age = 18.8 ± 1.4 years, height = 1.82 ± 0.08 m, weight = 75.1 ± 5.9 kg; mean ± SD) and 12 physically active control participants, who took part in recreational soccer no more than twice per week and had not played for a professional soccer club academy (CON; age = 22.2 ± 2.9 years, height = 1.75 ± 0.05 cm, weight = 71.6 ± 7.4 kg; mean ± SD), volunteered to participate in this study. Participants were classified as ESP if they competed at U18 and U23 level soccer in an English Premier League Category 1 soccer academy and were free from any lower limb injury. CON comprised recreationally active men, who were free from any lower limb musculoskeletal injury within the 3 months prior to the study. All participants provided written informed consent to take part in this study. Ethical approval was provided by Liverpool John Moores University Research Ethics Committee and complied with the Declaration of Helsinki.

4.2.2 Experimental Design

Participants attended the laboratory on two separate occasions, each one at least 48 hours apart, to complete a familiarisation session (60 minutes) and data collection session (120 minutes). The latter session was at least 48 h after any strenuous exercise. During the first session, participants were familiarised with the muscle function assessments, which comprised at least three plantar flexion (PF) and dorsiflexion (DF) maximal voluntary isometric contractions (MVCs) of the dominant leg (their preferred kicking leg) on an

isokinetic dynamometer. Following this, participants were familiarised with a series of bilateral and unilateral (dominant leg only) vertical and horizontal countermovement jumps (CMJs), as well as bilateral vertical drop jumps. During the second session, participant height and body mass were recorded, along with measurements of GM muscle architecture (fascicle length, L_f, and pennation angle, θ_p) and size (muscle thickness, MT, and anatomical cross sectional area, ACSA) using ultrasound. Muscle volume (V_m) and physiological cross-sectional area (PSCA) of the GM were assessed on a sub-group of 10 control participants. Participants were then positioned on an isokinetic dynamometer and performed a series of maximal PF and DF MVCs. Following this, participants performed a standardised warm-up protocol, comprising several dynamic movements and stretches, before completing two practice trials of each jump type. Participants performed three trials of each jump, using only their dominant leg, with their arms akimbo to minimise technique influencing performance. All jumps were performed using a force plate to record ground reaction force.

For more details of the specific procedures used to calculate GM morphology (including L_f, θ_p, ASCA, PCSA, V_m, and muscle thickness), PF and DF peak torque, and jump performance outputs (height, projectile range, peak power, peak force, reactive strength index, leg stiffness), please refer to chapter 3.

4.2.3 Statistical Analysis

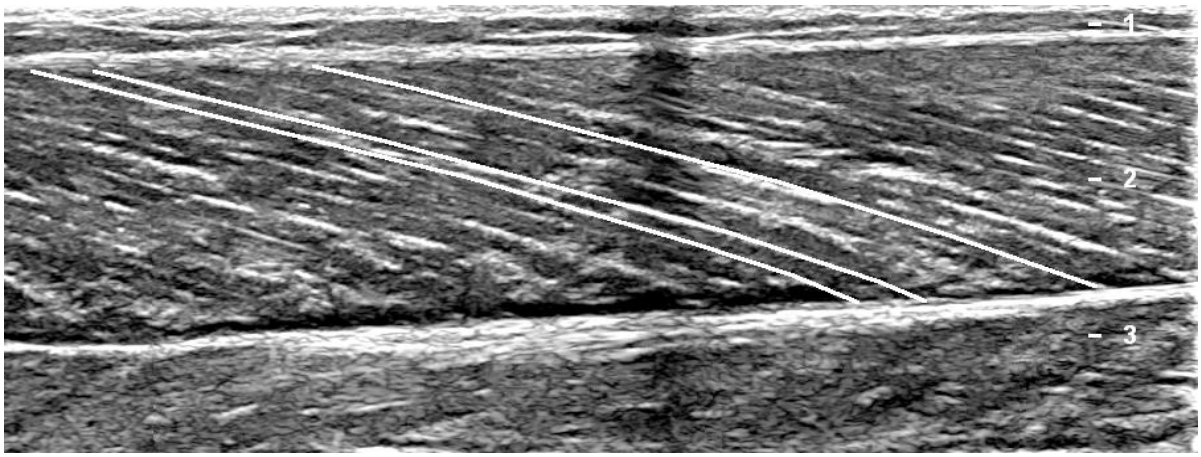
All variables were assessed to determine whether they met the parametric assumptions using a Levenes test for homogeneity of variance ($P<0.05$) and a Shapiro-Wilk test to determine normal distribution ($P<0.05$). If the data were parametric, differences between group means were determined using independent samples t-tests, however, if the data were non-parametric (e.g. not normally distributed), a Mann Whitney-U independent test was implemented instead. Similarly, Pearson's correlations were used to identify the relationship between neuromuscular characteristics and jump performance for parametric data, whilst Spearman's

correlations were used for non-parametric data. Descriptive statistics are presented as mean and standard deviation (SD) for all variables, unless otherwise stated. All statistical analyses were performed using SPSS (Version 26, IBM SPSS Inc., Chicago, USA) with statistical significance level set at $P<0.05$.

4.3 Results

Morphological characteristics of the GM muscle for ESP and CON are displayed in Table 1. Muscle θ_p was larger in ESP compared to CON ($P < 0.001$) but no other differences between groups were found.

A



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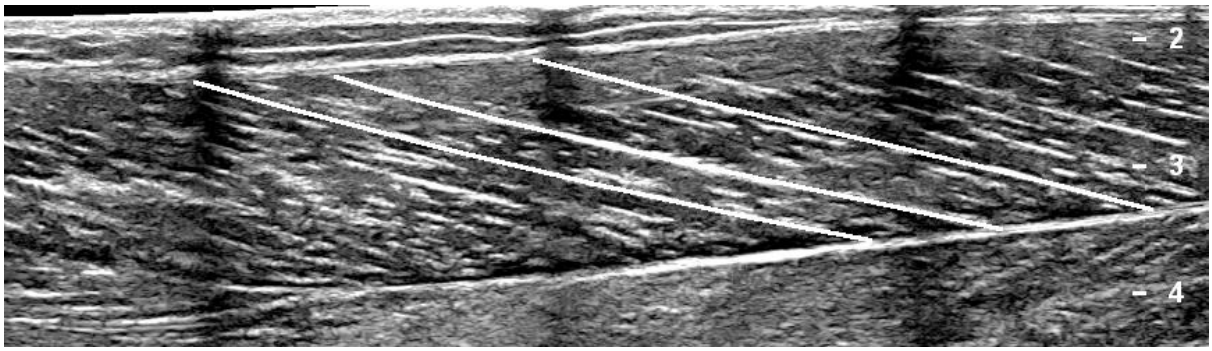


Figure 1. Representative muscle architecture ultrasound EFOV images at 50% GM muscle length from an ESP participant (**a**) and CON participant (**b**).

Table 1. Morphological characteristics of the GM in elite youth soccer players (ESP) and recreationally active controls (CON). Values are mean (SD).

Variable	ESP	CON	P Value
ACSA (cm^2)	14.3 (2.47)	13.9 (2.58)	0.296
L_f (cm)	5.91 (0.93)	6.24 (1.04)	0.352
θ_p (°)	17.4 (2.49)*	14.3 (1.23)	0.0002
MT (cm)	1.83 (0.21)	1.81 (0.24)	0.516

CSA, cross sectional area; L_f, fascicle length; θ_p, fascicle pennation angle; *MT*, muscle thickness; * different to CON.

Peak PF torque (209 vs 206 Nm) and peak DF torque (37 vs 34 Nm) were not different between ESP and CON ($P > 0.05$). Table 2 displays the between group differences for primary jump variables. Between group differences were found for peak horizontal power in bilateral and unilateral horizontal CMJ ($P \leq 0.007$). Projectile range was also greater in ESP ($P = 0.041$ and 0.006, respectively) for both bilateral and unilateral jumps, whilst no differences were found between groups for either the vertical CMJ or drop jumps.

Table 2. Jump variables for elite youth soccer players and recreationally active controls. Values are mean (SD).

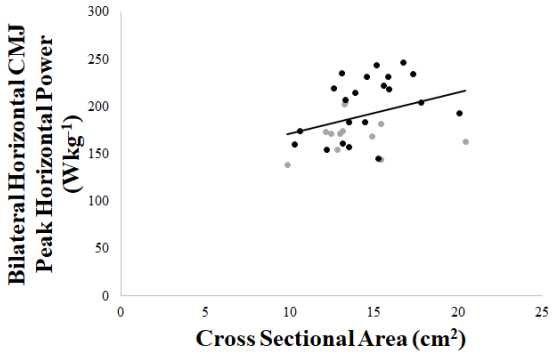
Jump	Variable (units)	ESP	CON
Bilateral VCMJ	Jump Height (cm)	43.9 (6.04)	40.7 (4.98)
	Peak Vertical Power (W kg^{-1})	240 (33)	230 (34)
Unilateral VCMJ	Jump Height (cm)	24.5 (2.9)	22.7 (7.1)
	Peak Vertical Power (W kg^{-1})	138 (34)	114 (30)
Bilateral HCMJ	Projectile Range (cm)	140 (14) *	129 (14)
	Peak Horizontal Power (W kg^{-1})	200.9 (32.6) **	170.7 (20.0)
Unilateral HCMJ	Projectile Range (cm)	103.9 (15.5) **	88.8 (11.8)

	Peak Horizontal Power (W kg^{-1})	145.6 (33.4) **	104.9 (15.1)
	Contact Time (s)	0.197 (0.017)	0.206 (0.017)
	Flight Time (s)	0.520 (0.047)	0.507 (0.026)
Bilateral Drop Jump	Jump Height (cm)	33.3 (5.90)	31.3 (3.39)
	Reactive Strength Index (AU)	2.65 (0.344)	2.48 (0.241)
	Vertical Stiffness (N·m·kg^{-1})	477.5 (196.5)	436.7 (217.9)

VCMJ, vertical countermovement jump; *HCMJ*, horizontal countermovement jump. * P < 0.05, ** P < 0.01.

Correlations were found between morphological characteristics and jump variables (Fig 1-5). Inverse correlations between L_f and unilateral VCMJ height (r = -0.529, P = 0.002) and peak power (r = -0.417, P = 0.016) were found, whilst positive correlations were found between the same jump variables and θ_p (r = 0.449 and 0.432, P = 0.009 and 0.012). ACSA at 50% GM length (r = 0.407, p = 0.019) and MT (r = 0.362, p = 0.038) correlated positively with peak horizontal power during a bilateral HCMJ. In the sub-sample of control participants (n=10), inverse correlations were found between V_m and bilateral VCMJ height (r = -0.840, P = 0.002), bilateral VCMJ peak power (r = -0.685, P = 0.029), bilateral HCMJ projectile range (r = -0.633, P = 0.05). No other significant correlations between GM morphological characteristics and jump variables were found.

A



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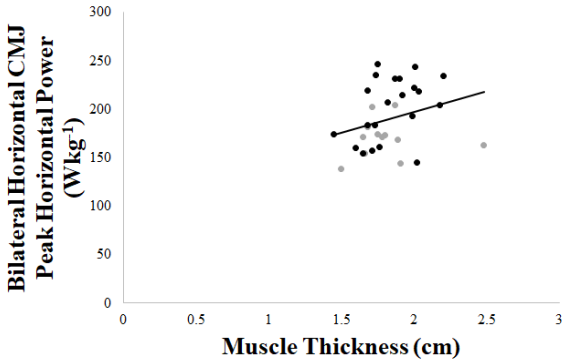


Figure 2. Relationship between bilateral horizontal countermovement jump (CMJ) horizontal peak power and GM CSA (**a**, r=0.407, P=0.019) and MT (**b**, r=0.632, P=0.038) in ESP (black data points) and CON (grey data points).

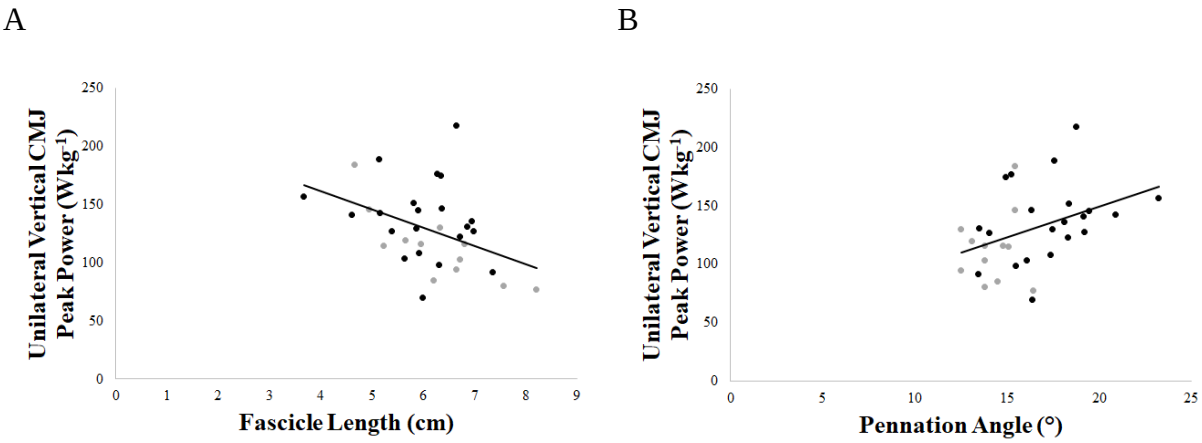


Figure 3. Relationship between unilateral vertical countermovement jump (CMJ) vertical peak power and GM L_f (**a**, r=-0.417, P=0.016) and θ_p (**b**, r=0.432, P=0.012) in ESP (black data points) and CON (grey data points).

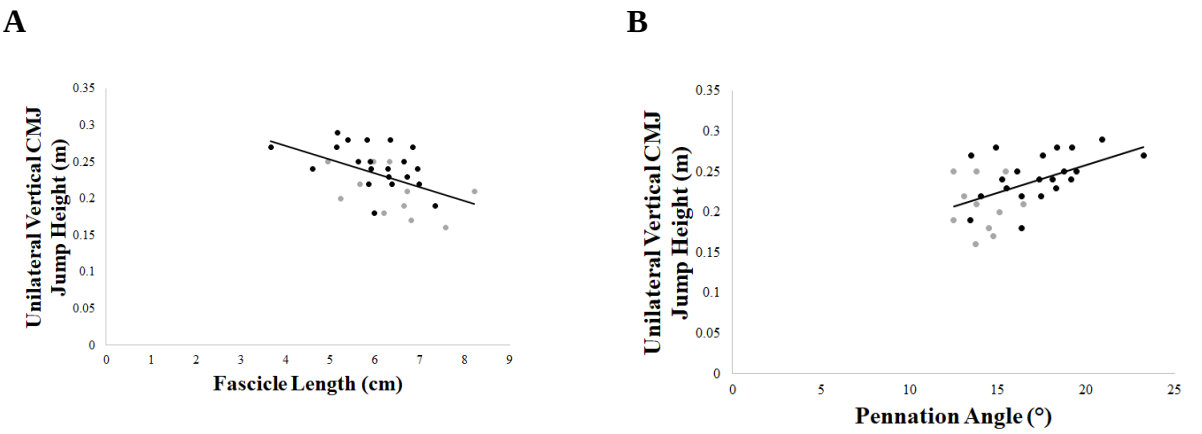
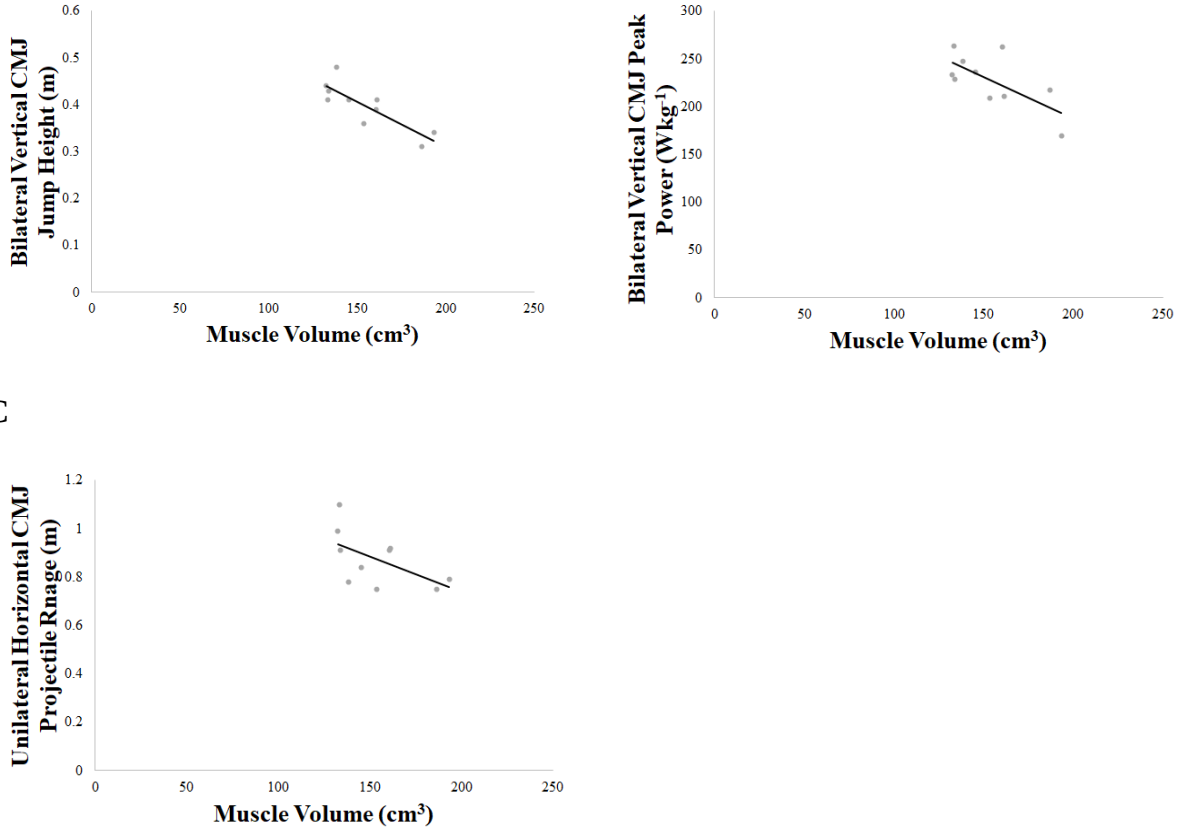


Figure 4. Relationship between unilateral vertical countermovement jump (CMJ) jump height and GM L_f (**a**, r=-0.529, P=0.002) and θ_p (**b**, r=0.449, P=0.009) in ESP (black data points) and CON (grey data points).

A **B**



C

Figure 5. Relationship between GM muscle volume and bilateral vertical countermovement jump height (**a**, r=-0.840, P=0.002), bilateral vertical countermovement jump peak power (**b**, r=-0.685, P=0.029) and bilateral horizontal countermovement jump projectile range (**c**, r=-0.633, P=0.05) (n=10) in CON only.

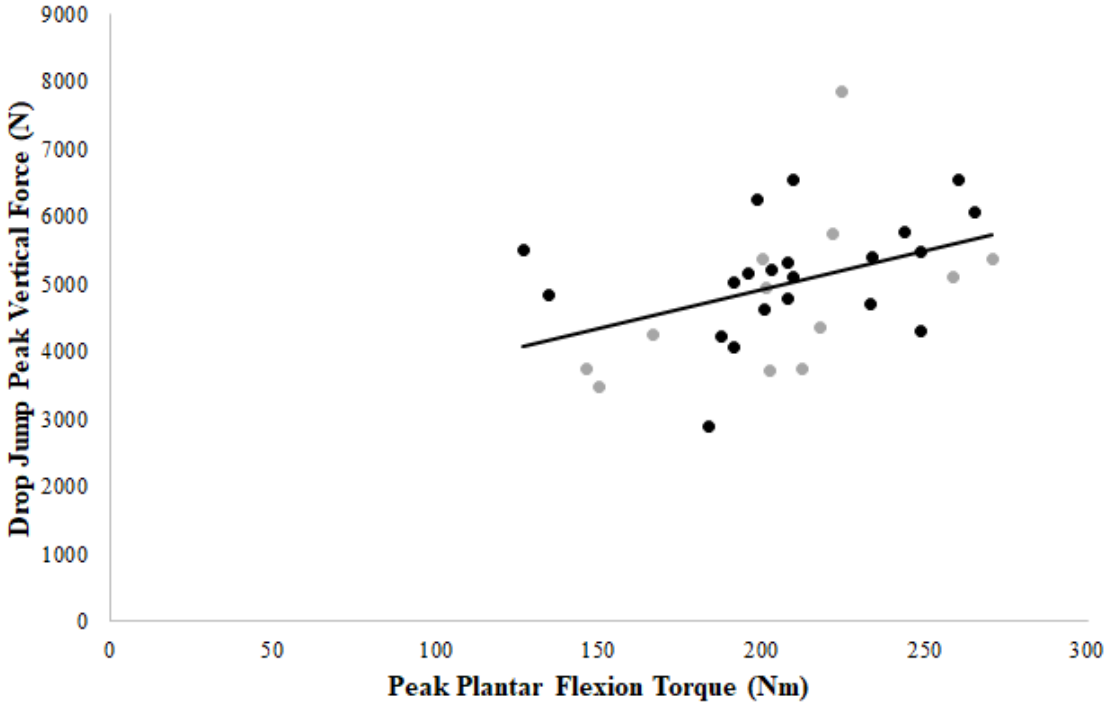


Figure 6. Relationship between bilateral drop jump peak force and plantar flexor peak torque (r=0.482, P=0.005) in ESP (black data points) and CON (grey data points). .

4.4 Discussion

The aim of this study was to investigate GM muscle strength and morphology in a group of ESP and CON participants, and to determine the relationship between these characteristics with markers of jump performance. The main findings were that ESP had a larger GM θ_p than CON and were capable of producing further distances with higher power outputs during horizontal jumping tasks. Furthermore, positive correlations between θ_p and performance in unilateral vertical CMJ were found, alongside inverse correlations for L_f with the same outputs. GM ACSA and MT both demonstrated positive relationships with peak horizontal power during a bilateral horizontal CMJ, whilst muscle volume correlated inversely with performance markers in bilateral vertical and horizontal CMJ in a smaller sub-group. These results suggest that a larger number of GM sarcomeres arranged in parallel rather than in

series is beneficial for augmenting power output and jump distance during unilateral vertical and bilateral horizontal CMJs.

Muscle architecture has been shown to differ between athletic populations (Kumagai *et al.*, 2000; Abe *et al.*, 2001; Kanehisa *et al.*, 2003; Aeles *et al.*, 2017), although limited information exists in the GM for ESP. The current values of GM muscle thickness, L_f and θ_p for both groups fall within the normative range reported previously for healthy, athletic populations (17-25 mm, 48-86 mm and 14-26 °, respectively), albeit towards the lower end (Kanehisa *et al.*, 2003; Karamanidis and Arampatzis, 2005; Stafilidis and Arampatzis, 2007; Mademli and Arampatzis, 2008; Karamanidis *et al.*, 2011; Baxter and Piazza, 2014; Geremia, Baroni, Lanferdini, *et al.*, 2018; Monte and Zamparo, 2019). Similarly, peak plantar flexion torque (173-228 Nm) fell within normative values previously reported for healthy adult males (Morse *et al.*, 2005; Wiesinger *et al.*, 2016; Hussain *et al.*, 2017). Similarities between ESP, CON individuals and previously reported control values might be surprising; however the ESP were not completing any plantar flexor specific training protocols at the time of testing, so limited adaptation might be expected to occur from soccer training.

GM muscle morphology did not differ between ESP and CON except that θ_p was greater in ESP. An increase in θ_p is thought to represent an increase in sarcomeres arranged in parallel, which will result in a hypertrophied fibre cross-sectional area (Aagaard *et al.*, 2001). In turn this provides a larger quantity of contractile tissue attaching to the aponeurosis, which provides a greater capacity to generate force (Kawakami, Abe and Fukunaga, 1993; Aagaard *et al.*, 2001). Differences in θ_p could therefore represent a training adaptation, induced by the habitually high joint and muscular forces imposed during pitch-based training, whereby consistent exposure to activities requiring large force production will likely result in architectural adaptation. Therefore, soccer training alone, without additional resistance

training intervention, may provide an effective stimulus for architectural adaptation in the GM.

However, training intervention studies have reported contrasting findings, as concentric-only, eccentric-only, conventional resistance and plyometric training, have all been shown to induce different architectural adaptations (Erskine *et al.*, 2011; Foure *et al.*, 2011; Franchi, Reeves and Narici, 2017; Geremia, Baroni, Lanferdini, *et al.*, 2018). Contraction-specific morphological adaptations have been summarised previously (Franchi, Reeves and Narici, 2017), with authors attributing differences in structural remodelling to molecular responses. However, the research in this area is still limited and it is unclear whether soccer-specific training elicits significant morphological adaptation of muscle and tendon and therefore requires further research.

Despite limited variation in GM architecture between our ESP and CON groups, several differences existed in the jump assessments. Peak horizontal power outputs during a horizontal CMJ (bilateral and unilateral) were greater for ESP, whilst no differences were found during vertical CMJ equivalent. These findings are in support of previous investigations, which suggest utilising horizontal jumps as a more informative assessment than vertical jumps (Meylan *et al.*, 2009; Murtagh *et al.*, 2017). Despite this, the power values attained in the present study are comparable to previous reports in groups of ESP for bilateral (220 $W \cdot Kg^{-1}$) and unilateral (144 $W \cdot Kg^{-1}$) vertical CMJ (Murtagh *et al.*, 2017). Murtagh *et al.*, (2017) also reported no group differences in jump height for both vertical jump types, although ESP did generate greater power in unilateral vertical CMJ compared to non-ESP. Whilst no statistically significant difference between ESP and CON was found in the current study (P=0.051), which contrasts with the findings of Murtagh *et al.*, (2017), jump performance variables were generally higher in the present CON compared to those reported

previously. These data could suggest an inherently better jumping ability in the CON participants of the present study.

Similarly, projectile range during unilateral horizontal CMJ was greater in the current ESP *vs.* CON, a finding which replicates that of Murtagh *et al.*, (2017). Limited data utilising projectile range or horizontal power to compare between athletic populations currently exist. Therefore, the current study is the first to demonstrate that horizontal power and projectile range can differentiate a group of ESP from recreationally active CON during a bilateral horizontal CMJ, in addition to a unilateral horizontal CMJ. Peak horizontal power outputs, when measured in absolute terms, i.e. as Watts rather than Watts per kilogram, are comparable to the values reported by (Meylan *et al.*, 2010) in a variety of team sport athletes (1029-1429 W *vs.* 988-948 W). Our findings might be slightly higher due to our homogenous athletic cohort comprising only ESP, which might invoke less variation from the mean. Further research should use projectile range and horizontal power from a bilateral and unilateral CMJ to compare between different athletic populations and as part of a testing battery to track time-course adaptations to soccer training interventions.

In contrast, none of the performance variables from a drop jump differed between ESP and CON. Values of RSI and vertical stiffness in the current study were higher than previously reported by Maloney, Richards and Fletcher (2018) (1.81 AU and 324.4 $N \cdot m^{-1} \cdot kg^{-1}$, respectively), who implemented a similar methodology. This difference, however, may be due to the population investigated, where Maloney, Richards and Fletcher (2018) recruited recreationally active males, whilst RSI values and drop jump parameters in other athletic populations are more in line with our findings (Markwick *et al.*, 2015; Douglas *et al.*, 2020). Nevertheless, a lack of observed difference between populations might be explained by the reliability of these outputs, as demonstrated in Chapter 3, where stiffness and RSI were found

to possess a between-session coefficient of variation of 18.7% and 10.2% and RLoA of ±65% and 39% respectively.

Whilst investigating potential differences in GM morphology and jumping ability between a group of ESP and CON was the primary aim of the present study, a second aim was to correlate the two variables to better understand the physiological determinants of jump performance. The findings from the present investigation accept the hypothesis that muscle size and θ_p would positively correlate with jump performance. Additionally, L_f displayed an inverse relationship with vertical jump height and vertical peak power.

Our positive correlations between θ_p and both jump height and power are in line with previous findings for the VL and LG (Earp *et al.*, 2010; Secomb, Nimphius, *et al.*, 2015; Secomb *et al.*, 2016; Murtagh, Nulty, *et al.*, 2018). Slightly stronger correlations were reported in the VL (r=0.478-0.58) (Secomb, Lundgren, *et al.*, 2015; Murtagh, Nulty, *et al.*, 2018), which might suggest a greater importance and contribution to power generation than either medial or lateral aspect of the gastrocnemius, which both appear to possess similar correlations with jump performance (r=0.432-0.449 and 0.460-0.469, respectively) (Earp *et al.*, 2010). Murtagh *et al.*, (2018) reported a positive correlation between VL θ_p and jump height in a unilateral vertical CMJ. Additionally, VL size measurements (volume, PCSA and thickness) correlated with peak vertical power in the same jump, stronger correlations being present for volume and PCSA, whilst no architectural measurements were associated with horizontal projectile range or horizontal power during a CMJ. Such findings suggest a direction-specific association between morphology and jump performance, whereby the ability to generate force, through greater PCSA, is a more integral component of power generation in the vertical, rather than the horizontal plane of motion. Similarly, in the LG, several authors have found positive relationships for θ_p with jump height, power and velocity (Earp *et al.*, 2010; Secomb, Nimphius, *et al.*, 2015; Secomb *et al.*, 2016). Larger θ_p, as

previously mentioned, is indicative of an improved ability to generate force, which might represent a similar mechanism for improvements in jumping performance to those observed through size measurements. This is of particular interest given that longer L_f has been reported in athletic populations and have shown to increase contractile velocity (Spector *et al.*, 1980; Sacks and Roy, 1982; Kumagai *et al.*, 2000; Abe *et al.*, 2001; Kanehisa *et al.*, 2003; Aeles *et al.*, 2017). Therefore, it might be surprising that an inverse correlation was found between GM L_f and jump height and power in unilateral vertical CMJ the present study. Taken together, our findings suggest that the number of sarcomeres arranged in parallel can influence the capacity for maximal force production to a greater extent than the number of serial sarcomeres. In support of this, Pentidis *et al.*, (2020) reported positive correlations between GM θ_p and maximal isometric ankle joint moment, which itself displayed a positive relationship with CMJ height. Therefore, plantar flexor force production would be essential for jump performance and θ_p is a dominant determinant.

Our findings might be explained by previous investigations into muscle-tendon interactions during a CMJ (Kurokawa, Fukunaga and Fukashiro, 2001; Kurokawa *et al.*, 2003). Changes to the muscle-tendon unit length during a countermovement jump are primarily explained by lengthening and shortening of the tendinous tissue (Kurokawa, Fukunaga and Fukashiro, 2001; Kurokawa *et al.*, 2003; Farris *et al.*, 2016). Rather than undergoing a stretch-shortening behaviour, muscle fibres remain close to their optimal length, contracting quasi-isometrically or shortening at slow contractile velocities (Kurokawa, Fukunaga and Fukashiro, 2001; Kurokawa *et al.*, 2003). The countermovement provides an opportunity to be in an active state for a longer duration, allowing for greater force to be produced (Bobbert *et al.*, 1996). Muscle θ_p and muscle size are already known determinants of a muscle's ability to generate force due to a larger quantity of contractile tissue available (Kawakami, Abe and Fukunaga, 1993; Fukunaga, Roy, *et al.*, 1996; Bamman *et al.*, 2000; Fukunaga *et al.*, 2001; Morse *et al.*,

2008; Blazevich *et al.*, 2009). Given that power is the result of force and velocity, it seems logical that architectural characteristics favouring force production would be associated with larger power outputs, particularly in the vertical plane. However, in contrast to this suggestion, inverse correlations from our smaller sub-sample of control participants (n = 10) were found between muscle volume and jump height/distance in VCMJ and HCMJ along with peak power in VCMJ. Interestingly, these relationships were found in bilateral jumping tasks, whilst the architectural correlations were with unilateral jumping variations. This could suggest that more contractile tissue is necessary to produce the required forces during a unilateral jump, when the demand to accelerate the centre of mass is expected to be greater. However, during the bilateral alternative, the same morphological characteristics could be detrimental when the required work is reduced.

To conclude, the current study demonstrates limited GM morphological differences exist between ESP and CON, although larger θ_p were found in ESP, which suggests soccer training induces an increase in the number of parallel-aligned sarcomeres. Correlations between architectural characteristics and jump performance outputs demonstrate positive relationships between θ_p and unilateral vertical CMJ performance, whilst L_f and V_m both correlated inversely with the same variables. GM ACSA and MT both correlated positively with horizontal peak power during a bilateral horizontal CMJ. Therefore, a greater number of sarcomeres arranged in parallel rather than in series would appear favourable for vertical and horizontal power output, potentially explained by an improved ability to generate greater GRFs. That being said, it is important to understand how the demands of soccer training affect the architectural characteristics of ESP and physical performance to help practitioners form training interventions to support physical development. Currently, the role of soccer training as a stimulus for this adaptation is unknown and requires further investigation.

Chapter Five

Gastrocnemius medialis tendon properties do not differ between elite youth soccer players and control participants but are related to jump performance

Prelude

Similar to the investigation in chapter 4, this chapter will be examining the series elastic element (SEE) of the GM MTU, utilising the techniques described in detail in chapter 3. The literature review in chapter 2 suggests there are distinctive functions of the muscle and tendon during force and power production and each element is responsible for a different action. Whilst the contractile element (CE) is responsible for generating large forces, as demonstrated in chapter 4, the tendon is expected to be accountable for the large recoil velocities required, as per the catapult effect, to amplify power output. However, the physiological characteristics that influence this function, along with the influence this may have on power and reactive strength, are currently unknown. Therefore, this chapter seeks to provide an insight into the relationships between the GM mechanical and material tendon properties and performance in jumping tasks.

Abstract

The aims of this study were to investigate (i) differences in gastrocnemius medialis (GM) tendon properties between elite youth male soccer players (ESP) and controls (CON); and (ii) the relationships between GM tendon properties and jump performance. The GM tendon force-elongation relationship was measured in 13 ESP (mean ± SD height (m); mass (kg), age (years): 1.81 ± 0.07 m; 73.6 ± 5.4 kg; 18.8 ± 1.2 years) and 11 male CON (1.74 ± 0.05 m; 71.2 ± 7.6 kg; 22.3 ± 3.0 years) using a combination of dynamometry, ultrasonography and electromyography. Participants also performed a series of unilateral and bilateral countermovement jumps (CMJ) and bilateral drop jumps on a force platform. GM tendon properties did not differ between groups but unilateral vertical CMJ peak vertical power correlated inversely with maximal tendon elongation (r=-0.431, P=0.035) and strain (r=-0.431, P=0.036), while tendon length correlated with peak horizontal power during a unilateral horizontal CMJ (r=0.359, P=0.040). Furthermore, vertical stiffness and peak force during a drop jump correlated with tendon stiffness (r=0.431-0.462, P=0.035-0.023), maximal tendon force and tendon CSA (r=0.409-0.737, P≤0.047). These results suggest that GM tendon properties do not differ between ESP and CON but a stiffer GM tendon may facilitate the production of larger forces to increase power output during a unilateral vertical CMJ. Furthermore, higher force and vertical stiffness outputs are achieved during a drop jump by stronger individuals with stiffer and larger GM tendons, which could represent a protective mechanism to shield the tendon against damage.

5.1 Introduction

Maximal acceleration and sprinting are considered to be key performance indicators (KPIs) for soccer players (Faude, Koch and Meyer, 2012; Emmonds *et al.*, 2016). Jump assessments, such as countermovement jumps (CMJ) and drop jumps, are frequently used to isolate physical qualities required for acceleration and sprinting (power and stiffness), due to eliciting similar muscle-tendon unit (MTU) behaviour (Kurokawa, Fukunaga and Fukashiro, 2001; Kurokawa *et al.*, 2003; Ishikawa, Niemelä and Komi, 2005; Sousa *et al.*, 2007; Maloney, Richards and Fletcher, 2018; Murtagh, Nulty, *et al.*, 2018). Tendons are integral to jumping and sprinting performance, with great demands placed on the gastrocnemius medialis (GM) MTU to generate and transmit large forces in short time frames (Lai *et al.*, 2018; Schache *et al.*, 2019). The Achilles tendon is central to the large angular velocities generated around the ankle joint, created by a 'catapult' effect due to the relatively slow fascicular shortening occurring in the GM and soleus (Bobbert, Huijing and van Ingen Schenau, 1986a, 1986b; Kurokawa, Fukunaga and Fukashiro, 2001; Kurokawa *et al.*, 2003).

Furthermore, tendons are highly plastic tissues and can adapt to the load placed on them during habitual activity. Changes in tendon elasticity following a period of chronic resistance training have been shown to influence its ability to store, release and transfer force (Bojsen-Møller *et al.*, 2005; Fouré, Nordez and Cornu, 2010; Waugh *et al.*, 2013), which in turn can impact behaviour during dynamic tasks (Lichtwark and Wilson, 2008; Werkhausen *et al.*, 2018). Cross-sectional research has demonstrated that elite athletes have different tendon properties in the knee extensors compared to non-elite athletes (Kubo *et al.*, 2000; Stafilidis and Arampatzis, 2007; Wiesinger *et al.*, 2016; Murtagh, Stubbs, *et al.*, 2018), where such differences have been attributed to the loading parameters induced through their respective sports, which may also influence physical performance during sport-specific tasks.

However, whilst patellar tendon research has been used to differentiate between athletic populations previously, research surrounding the GM tendon is less clear and often produces conflicting results. Differences in Achilles tendon morphological and mechanical properties between athletic populations have been observed previously (Arampatzis *et al.*, 2007; Wiesinger *et al.*, 2016), which contrasts with other reports in youth and adult athletes (Rosager *et al.*, 2002; Karamanidis and Arampatzis, 2006; Stafilidis and Arampatzis, 2007; Kubo, Tabata, *et al.*, 2010; Kubo *et al.*, 2017; Pentidis *et al.*, 2019). However, the majority of this research has taken place in running-based populations, while to the best of the author's knowledge, only shear wave and myotonometric methods have been utilised to assess tendon properties in soccer players (Cristi-Sánchez *et al.*, 2019; Minafra *et al.*, 2020). Due to the inherently different loading patterns between soccer and track running, it could be expected that the GM tendon would exhibit differing mechanical and material properties between athletic populations. Furthermore, given recent observations in the patellar tendon (Murtagh, Stubbs, *et al.*, 2018), investigation is required into the properties of the GM tendon in elite youth soccer players to determine if this tendon is equally as important in distinguishing athletes from this population compared to age-matched control participants.

In addition to population-specific properties, authors have previously sought to understand how tendon mechanical properties, measured in vivo, can relate to jumping performance (Kubo *et al.*, 2000; Foure *et al.*, 2011; Abdelsattar, Konrad and Tilp, 2018; Murtagh, Stubbs, *et al.*, 2018). In particular, research investigating the patellar tendon has suggested a more compliant tendon is beneficial for jumping performance involving a countermovement (Kubo, Kawakami and Fukunaga, 1999; Kubo *et al.*, 2006; Murtagh, Stubbs, *et al.*, 2018). However, the relationship between GM tendon properties and jump performance is inconclusive due to contrasting findings (Burgess *et al.*, 2007; Kubo, Morimoto, Komuro,

Tsunoda, *et al.*, 2007; Fouré *et al.*, 2009; Wu *et al.*, 2010; Abdelsattar, Konrad and Tilp, 2018; Pentidis *et al.*, 2020).

Several authors have demonstrated positive correlations between GM tendon stiffness and jump performance, although this may depend on the type of jump employed (Burgess *et al.*, 2007; Kubo, Morimoto, Komuro, Tsunoda, *et al.*, 2007; Wu *et al.*, 2010; Pentidis *et al.*, 2020). A purely concentric jump, or drop jump, might rely on a faster transmission of force to the bone, which can be facilitated by a stiffer tendon (potentially increasing the rate of force development) (Bojsen-Møller *et al.*, 2005; Burgess *et al.*, 2007; Kubo, Morimoto, Komuro, Yata, *et al.*, 2007a; Waugh *et al.*, 2013; Hirayama *et al.*, 2017; Abdelsattar, Konrad and Tilp, 2018). However, increases in GM tendon stiffness, following isometric training interventions, are not necessarily concurrent with improvements in jump height during a CMJ or drop jump (Burgess *et al.*, 2007; Kubo, *et al.*, 2007a). Rather, jump heights can increase following plyometric training in the absence of changes to the tendon stiffness (Kubo, *et al.*, 2007b; Fouré *et al.*, 2009). Therefore, inconsistent findings between altered mechanical properties of the GM tendon and changes in jumping performance outputs have led to uncertainty in the relationship between the two variables and therefore require further investigation.

Understanding how GM tendon characteristics might influence performance in KPIs may help with the design of training interventions. Therefore, the aims of this study were to i) investigate the mechanical and material properties of the GM tendon in a group of elite youth male soccer players (ESP) and age-matched recreationally active male controls (CON); and ii) investigate relationships between these properties and performance in a series of jumping tasks. It was hypothesised that GM tendon stiffness would be greater in ESP *vs.* CON, and would correlate positively with jump performance during CMJs and drop jumps.

5.2 Methods

5.2.1 Participants

Twenty-four healthy young men, comprising 13 elite youth soccer players (ESP; age, 19 ± 1 years; height, 1.81 ± 0.07 m; mass, 73.6 ± 5.4 kg; mean ± SD), and 11 recreationally active control participants (CON; age, 22 ± 3 years; height, 1.74 ± 0.05 m; mass, 71.2 ± 7.6 kg) volunteered to take part in this study, which was approved by Liverpool John Moores University Research Ethics Committee and complied with the Declaration of Helsinki. ESP all participated in U18 and U23 level football in an English Premier League Category 1 soccer academy, while CON were included if they undertook 3-4 hours per week of recreational sporting activities, including soccer training. All participants were free from any lower limb injury within the past 3 months and provided written informed consent to participate in this study.

5.2.2 Experimental Design

Participants attended the laboratory on two separate occasions at least 48 hours apart. The initial session familiarised participants with all relevant testing procedures, which included at least 3 plantar flexion (PF) and dorsiflexion (DF) maximal voluntary isometric contractions (MVCs) of the dominant leg (their preferred kicking leg). Additional 6 s ramped PF MVCs were performed. After this, participants were familiarised with a series of bilateral and unilateral (dominant limb only) vertical countermovement jumps, horizontal countermovement jumps and (bilateral only) vertical drop jumps. Participants performed at least 3 trials for each jump protocol. During the second session (at least 48 h post intensive activity), with the participants relaxed in a prone position with their ankle at 0° (neutral), ultrasound images were taken in the sagittal plane along the length of the gastrocnemius (GM) tendon and transverse images at 1 cm intervals along the Achilles tendon from the distal osteotendinous junction were also captured. Participants were then seated on an

isokinetic dynamometer and produced 3 PF MVCs, 3 DF MVCs, 3 x 6 s ramped PF MVCs and several passive ankle rotations with an ultrasound probe positioned over the distal GM myotendinous junction (MTJ). Surface EMG electrodes recorded agonist (GM) and antagonist (tibialis anterior, TA) muscle activity during all MVCs. Participants then performed a standardised warm up, which involved jogging, dynamic movements and stretches, before completing three practice trials of each jump type. Following this, participants completed three trials of each jump protocol on a force plate, recording vertical, medio-lateral, and anterior-posterior ground reaction forces.

For more details of the specific methods used to calculate mechanical and material properties of the GM tendon (cross-sectional area, tendon length, moment arm maximal elongation, stiffness, strain, stress and Young's modulus) and performance variables from jump assessments (height, power, projectile range, stiffness, peak force), please refer to Chapter 3. Specifically, Method 1 was used to calculate the GM tendon force-elongation relationship and all derivatives thereof.

5.2.3 Statistical Analysis

All variables were assessed to determine whether they met the parametric assumptions using a Levenes test for homogeneity of variance ($P<0.05$) and a Shapiro-Wilk test to determine normal distribution ($P<0.05$). If the data were parametric, differences between group means were determined using independent samples t tests. If data were non-parametric (e.g. not normally distributed), a Mann Whitney-U independent test was used to compare between group differences. To compare between (ESP vs CON) and within (location) group differences in tendon CSA, a mixed ANOVA was implemented. Pearson's correlations were used to identify the relationships between mechanical tendon properties and jump performance. Descriptive statistics are presented as mean and standard deviation (SD) for all

variables, unless otherwise stated. All statistical analyses were performed using SPSS (Version 26, IBM SPSS Inc., Chicago, USA) with statistical significance level set at $P<0.05$.

5.3 Results

Mechanical and material properties of the GM tendon for the elite youth soccer players (ESP) and recreationally active control (CON) participants are displayed in Table 1. Results from the mixed ANOVA for tendon CSA are presented in Figure 1; no significant differences were observed between- or within-groups at any location. In fact, no differences were observed between the two groups for any tendon variable ($P>0.05$).

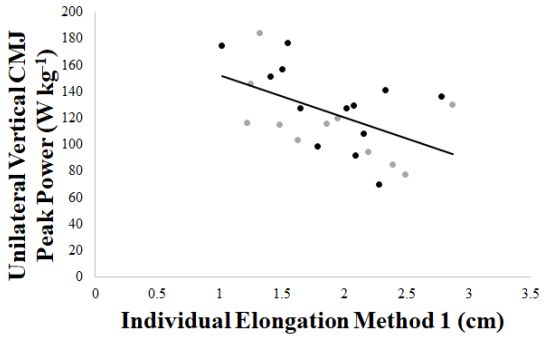
Table 1. Comparison of GM tendon morphological, mechanical and material properties in elite youth soccer players (ESP) and recreationally active control participants (CON). Values are mean (SD).

Variable (units)	**ESP (n= 13)**	**CON (n = 11)**
1 cm CSA (mm^2)	76.7 (13.1)	73.6 (11.0)
2 cm CSA (mm^2)	73.5 (13.1)	68.0 (7.6)
3 cm CSA (mm^2)	66.3 (10.2)	61.8 (7.3)
4 cm CSA (mm^2)	61.6 (10.6)	58.9 (8.3)
Mean CSA (mm^2)	69.6 (10.8)	65.6 (7.8)
GM Tendon Length (cm)	20.8 (2.4)	19.4 (2.0)
Moment Arm (mm)	30.9 (5.4)	34.2 (4.8)
Maximum Tendon Force (N)	1334 (235)	1260 (307)
Individual Stiffness (N/mm)	903 (405)	941 (431)
Common Stiffness (N/mm)	824 (223)	789 (260)
Individual Elongation (cm)	1.90 (0.47)	1.88 (0.56)
Common Elongation (cm)	1.14 (0.29)	1.31 (0.43)
Individual Strain (%)	9.15 (2.36)	9.70 (3.09)
Common Strain (%)	5.47 (1.36)	6.80 (2.40)
Individual YM (GPa)	1.42 (0.83)	1.32 (0.49)
Common YM (GPa)	1.28 (0.52)	1.12 (0.26)
Individual Stress (MPa)	19.7 (3.8)	19.2 (4.04)
Common Stress (MPa)	11.2 (1.7)	11.7 (1.43)

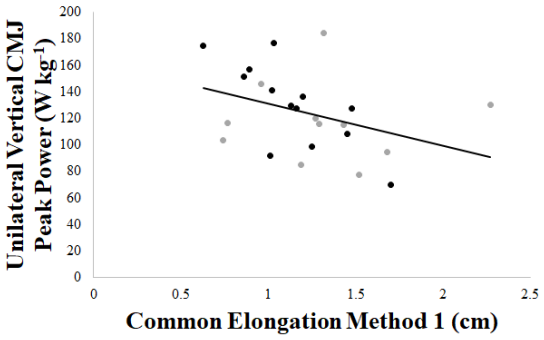
CSA, cross-sectional area; *YM*, Young's modulus; *Individual*, maximum value for that variable; *Common*, value for that variable measured at the highest common force.

Correlations between mechanical properties of the GM tendon and jump performance variables are presented in Figure 1-3. Inverse correlations were found for peak vertical power during a unilateral CMJ with individual and common elongation characteristics, along with individual strain, force and stress properties. Similarly, unilateral vertical CMJ height correlated inversely with both methods of calculating individual strain properties. Vertical stiffness during a drop jump correlated positively with common stiffness, maximum tendon force and common stress values.

A



B



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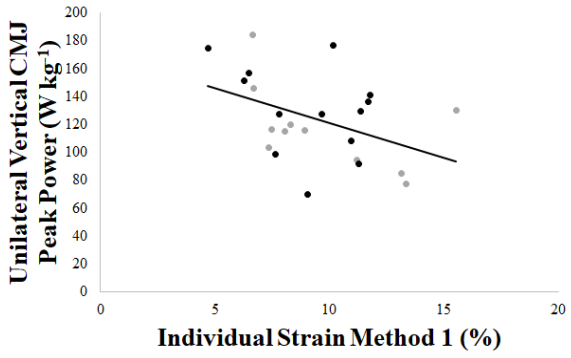

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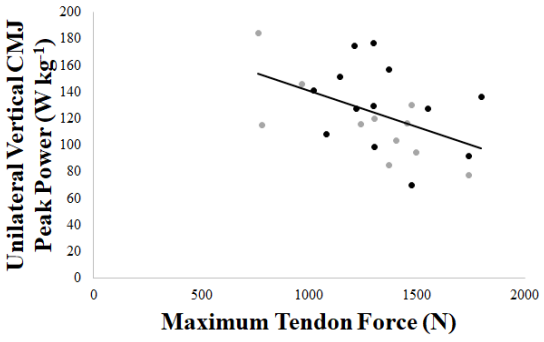

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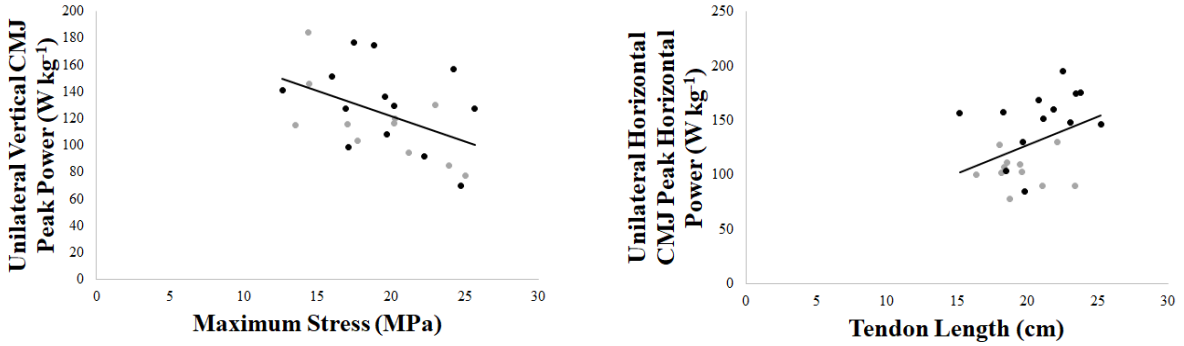


Figure 1. Relationship between unilateral vertical countermovement jump (CMJ) vertical peak power and maximal tendon elongation (**a**, r = -0.515, P=0.010), tendon elongation over a common force range (**b**, r = -0.415, P=0.044), maximal tendon strain (**c**, r = -0.431, P=0.036), maximum tendon force (**d**, r = -0.474, P=0.019); individual stress (**e**, r = -0.469, P=0.021); and the relationship between unilateral horizontal countermovement jump (CMJ) horizontal peak power and GM tendon length (**f**, r = 0.359, P=0.040) in ESP (black data points) and CON (grey data points).

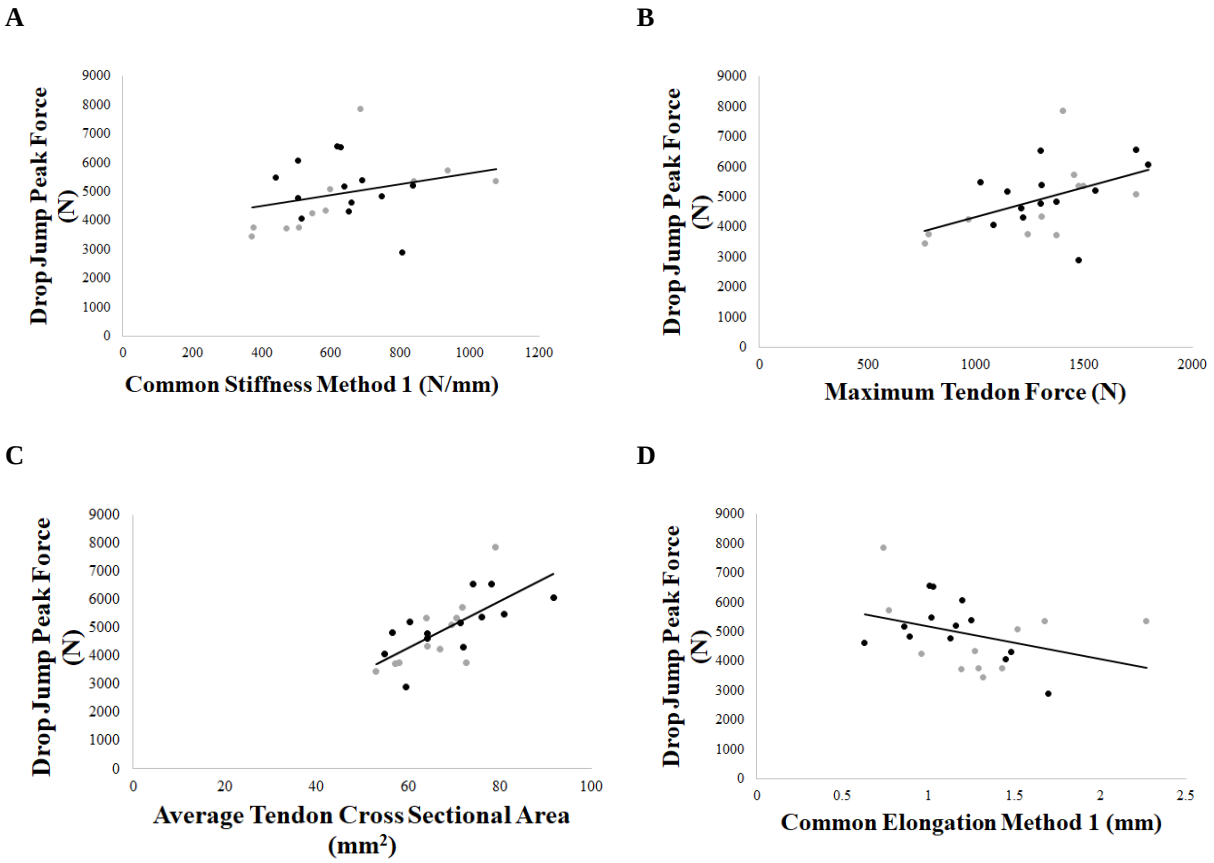


Figure 2. Relationship between vertical drop jump peak force and stiffness over a common force range (**a**, r = 0.431, P =0.035), maximal individual force (**b**, r = 0.501, P=0.013), average tendon cross-sectional area (CSA) (**c**, r = 0.737, P <0.001) and elongation over a common force range (**d**, r = -0.416, P=0.043) in ESP (black data points) and CON (grey data points).

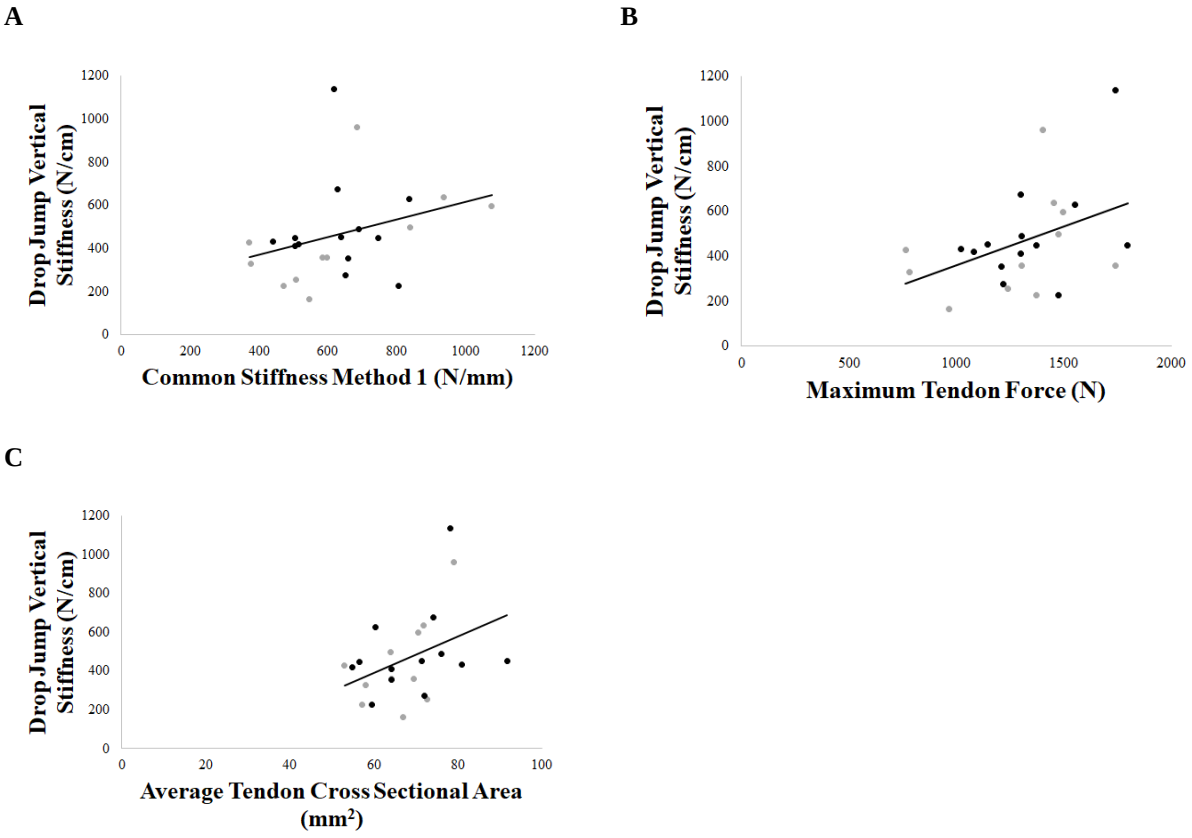


Figure 3. Relationship between vertical drop jump stiffness and stiffness over a common force range (**a**, r = 0.462, P=0.023), maximal individual force (**b**, r = 0.441, P=0.031) and average tendon cross-sectional area (CSA) (**c**, r = 0.409, P =0.047) in ESP (black data points) and CON (grey data points).

5.4 Discussion

The aims of this study were to investigate whether (i) the mechanical and material properties of the GM tendon differed between a group of ESP and CON; and (ii) GM tendon properties

were related to power and force variables during different CMJs and drop jumps. The current study demonstrated that GM mechanical and material properties did not differ between ESP and CON. However, several key correlations between tendon properties and jump performance were found, whereby maximal elongation and strain of the GM tendon correlated inversely with unilateral CMJ vertical peak power, while tendon stiffness, maximum force and CSA all correlated positively with peak force and vertical stiffness during a bilateral drop jump. This study has therefore identified key properties of the GM tendon that could be required to tolerate higher GRFs and satisfy greater stiffness requirements.

Morphology of the GM tendon (CSA and length) for both groups fell within normative values previously reported for healthy adult men (50-78 mm^2 and 18-24 cm, respectively) (Magnusson *et al.*, 2001; Magnusson and Kjaer, 2003; Kongsgaard *et al.*, 2011; Stenroth *et al.*, 2012; Wiesinger *et al.*, 2016; Geremia, Baroni, Lanferdini, *et al.*, 2018; Mogi *et al.*, 2018; Monte and Zamparo, 2019). Similarly, Achilles tendon moment arm was also in line with data previously reported utilising the tendon excursion method (29-36 mm) (Fath *et al.*, 2010, 2013; Baxter and Piazza, 2018; Buffey *et al.*, 2020), although this method is known to report lower values compared to the centre of rotation method using magnetic resonance imaging (Fath *et al.*, 2010) or dual-energy X-ray absorptiometry (Buffey *et al.*, 2020).

Maximal strain and elongation values were similar (6-9 % and 16-18 mm, respectively) to values reported by studies utilising a similar methodology, measuring tendon displacement at the GM MTJ whilst participants were seated on an isokinetic dynamometer (Waugh *et al.*, 2012; Wiesinger *et al.*, 2016; Geremia, Baroni, Bobbert, *et al.*, 2018). However, lower strain values have been reported previously (Kubo *et al.*, 2014, 2015), which can be attributed to either recording the aponeurosis displacement rather than the MTJ, or through positioning participants prone during MVCs. These discrepancies could be caused by greater tendon

elongation at proximal locations (i.e. aponeurosis), resulting in reduced overall strain for a given elongation (Muramatsu, Muraoka, Takeshita, Kawakami, Hirano, Fukunaga, *et al.*, 2001; Kubo *et al.*, 2002; Arampatzis *et al.*, 2005). Similarly, the improved ability to generate greater PF torque when seated as opposed to prone (188-228 and 118-171 Nm, respectively) (Kubo *et al.*, 2014, 2015, 2017; Wiesinger *et al.*, 2016; Monte and Zamparo, 2019) could impose a larger stretch on the tendon, thus allowing it to elongate further and recording higher values of mechanical strain.

Regardless of the methodological differences between studies, the lack of difference in GM tendon strain or elongation parameters between ESP and CON in the current study is in agreement with earlier studies, investigating GM properties in different populations. Previous investigations report a lack of difference in GM tendon strain between athletes with different habitual loading patterns (Arampatzis *et al.*, 2007; Kubo *et al.*, 2015; Wiesinger *et al.*, 2016), suggesting elongation and strain might not change in response to athletic activities, although soccer players have not been investigated previously. In support of this, no change in GM tendon strain or elongation has been reported following a range of training interventions (Foure *et al.*, 2011; Kubo, Ishigaki and Ikebukuro, 2017; Geremia, Baroni, Bobbert, *et al.*, 2018).

Furthermore, no differences between ESP and CON groups were found for tendon stiffness or Young's modulus. However, due to the wide variety of approaches taken to determining stiffness within the literature, comparison across studies is difficult. Nevertheless, few authors have reported differences in GM tendon stiffness between athletic populations. Arampatzis *et al.*, (2007) reported greater GM tendon stiffness in sprinters compared to endurance runners and non-active individuals but did so using the force-strain relationship as opposed to the force-elongation to circumvent issues arising from heterogeneous elongation along the length of the tendon-aponeurosis. However, these higher stiffness values coincided

with larger plantar flexion forces in the sprinting group, whereby 67% of the variance in tendon stiffness was associated with maximal tendon force. This suggests that the changes in stiffness were the result of a coordinated MTU adaptation in response to increases in muscle strength and represent a change to the intrinsic properties of the tendon to cope with the increased force generating ability. In the present study, this could explain why no difference in tendon properties were found between ESP and CON, given the closeness of plantar flexor strength and maximal tendon force between the two groups. The similarity in peak torque values could demonstrate the limited ability of soccer training in elicit improvements in neuromuscular strength, although it is not yet known if this is the case (but this will be investigated in the subsequent chapter).

Training interventions have demonstrated the plasticity of tendons in response to high loading strategies, which can alter their ability to store, release and transfer energy (Kubo, Morimoto, Komuro, Yata, *et al.*, 2007; Foure *et al.*, 2011; Geremia, Baroni, Bobbert, *et al.*, 2018). However, whether these adaptations are favourable for jump performance remains unclear. As mentioned in chapter 3, the physical characteristics identified during the jumping task (i.e. power and stiffness), rather than the height or distance attained, should be the focus of the assessment. Previous studies have investigated the relationship between tendon properties and jump height, with contrasting findings, but few studies have examined correlations with power or stiffness outputs (Burgess *et al.*, 2007; Kubo, Morimoto, Komuro, Tsunoda, *et al.*, 2007; Wu *et al.*, 2010; Pentidis *et al.*, 2020). In the current study, inverse correlations were found between peak power output during a unilateral vertical CMJ and GM tendon elongation and strain. Such findings may suggest that a stiffer GM tendon is able to assist in generating larger power outputs. Maximal elongation can depend on the resting length of a tendon, but similar relationships with performance were still found for strain measurements.

Given that power is the product of force and velocity, a less extensible tendon would likely coincide with either a greater ability to generate force or an improved ability to recoil at faster velocities. Recent findings by Werkhausen *et al.*, (2019), demonstrated that, despite an increase in stiffness following resistance training, Achilles tendon recoil velocity during running reduced. However, this reduction was present despite no change in strain of the Achilles tendon, which then suggests that for a less extensible tendon to maintain a stretch equal to that of a more compliant tissue, additional musculoskeletal mechanisms must be altered. Given the findings of the present study, it would therefore be logical to assume that for a given strain to be achieved in a stiffer tendon, a larger production of force would be needed than required for a more compliant tendon. The energy stored and released by the stiffer tendon would therefore be greater, which in turn would return a more forceful MTU contraction, increasing power output. This proposition would be in support of the findings from chapter 4, which suggested that an individual with a greater capacity to generate force through morphological parameters (i.e. muscle size) would have an improved ability to generate power during a unilateral vertical CMJ. Alternatively, a stiffer tendon may undergo less elongation during stretch (Hicks *et al.*, 2013), causing the attaching muscle to contract eccentrically. This eccentric contraction could generate a larger force and in turn, amplify power output during the concentric phase of the jump. Whilst Werkhausen *et al.*, (2019) reported no change in force production during running following resistance training, the authors found an increase in GM architectural gear ratio, which may provide an alternative mechanism for maintaining the strain of a stiffer tendon. During contraction fascicles are known to shorten and rotate, thus leading to greater shortening of the overall muscle than the individual fascicle shortening (Maganaris, Baltzopoulos and Sargeant, 1998; Azizi, Brainerd and Roberts, 2008). An increase in this ‘gearing’ of the muscle fascicle will lead to an increased stretch placed on the tendon, allowing strain to be maintained in a stiffer tendon.

Regardless of the specific mechanisms that influence tendon strain during running and jumping, a stiffer tendon would still be expected to augment power output.

However, the inverse relationships between power output and tendon elongation and strain conflict with previous reports of the patellar tendon, where a more compliant tendon has been associated with power output in similar jumps assessments (Murtagh, Stubbs, *et al.*, 2018). These findings might represent the innate differences between these two MTUs, as the quadriceps MTU possesses a greater quantity of contractile tissue than the plantar flexors (Wickiewicz *et al.*, 1983), coupled with a shorter, thicker tendon. Whilst power and positive work generated by the GM MTU during running and jumping requires a larger contribution from the tendon than muscle fibres (Bobbert, Huijing and van Ingen Schenau, 1986b; Lai *et al.*, 2014), the quadriceps might require more contribution from the contractile tissue. A more compliant patellar tendon could therefore facilitate the generation of greater forces during CMJ.

In addition, the lack of correlation between GM tendon properties and power generated during horizontally oriented jumps might be the influence of altered coordination patterns and work completed by the plantar flexors (Nagano, Komura and Fukashiro, 2007). Longer times to take-off following the onset of movement may dampen the contribution of the tendon, relying more on muscular contribution for power production. This is supported by our findings in chapter 4, which demonstrated positive correlations between peak horizontal power and measures of muscle size.

It might be surprising that tendon stiffness has not also shown the same relationship as strain, given stiffness is the result of changes in length relative to changes in force during the linear region of the force-elongation relationship. Our findings suggest the ability of the tendon to elongate over these maximal force ranges does not influence power output during a vertical

CMJ. Previous research has assessed tendon length change over 50-100% MVC (Kubo, Tabata, *et al.*, 2010; Kubo *et al.*, 2014; Fletcher and MacIntosh, 2015; Kubo *et al.*, 2015, 2017), which may provide some variance to the present investigation which used 80-100% MVC. A higher force range would likely isolate the linear region, but may miss some significant length changes to the tendon. Should variation be present between populations, a narrow force-range might not subtle enough to detect such differences.

Stiffness over a common force range did, however, display a positive relationship with vertical GRF and leg stiffness during a bilateral drop jump. Additional positive relationships were observed between maximal tendon force and vertical GRF and leg stiffness, along with a positive correlation between tendon CSA and peak vertical GRF. It should not be surprising that all three tendon properties (stiffness, CSA and force) displayed similar correlations with GRF, given the close association between them. Force and stiffness have regularly displayed a linear relationship with one another (Arampatzis, Mersmann and Bohm, 2020), whilst a larger CSA would likely be required to coincide with a greater capacity of the muscle to produce force to increase the safety factor of the tendon and reduce the risk of rupture (Magnusson and Kjaer, 2003). Therefore, it could be expected that relationships for one of these variables would co-exist with similar correlations for the other parameters. Similarly, given that vertical stiffness is the product of the centre of mass displacement and force generated, comparable relationships might be expected for these three parameter for a given mechanical property.

One possible explanation for the positive relationships observed could be that a stiffer tendon enables muscle fibres to remain closer to isometric during contraction, as previously suggested by Hirayama *et al.*, (2017). Less displacement of the tendon for a given force would reduce the fascicular shortening required, thus enabling larger forces to be generated from a more optimal length-tension position. Alternatively, an eccentric contraction of the

GM may also allow less displacement of the tendon, whilst generating higher forces to be returned via elastic energy. Whilst previous research has suggested positive correlations between Achilles tendon stiffness and contact time during a drop jump (Abdelsattar, Konrad and Tilp, 2018), the current study found no such relationship. Instead, these findings might be more closely related to greater strength and muscle activity capabilities, although increases in EMG activity might not always correlate with changes in jump performance (Kubo, *et al.*, 2007a). Additionally, positive correlations between jump performance and tendon CSA suggest that a larger tendon would be able to withstand higher forces produced by the GM, increasing its safety factor, all of which serve to augment vertical GRF and in turn, vertical stiffness, during a drop jump. In support of this suggestion, Monte and Zamparo, (2019) showed an association between Achilles tendon CSA and sprint performance, suggesting an increase in tissue quantity has an augmented effect on performance, possibly able to transmit larger forces more effectively. Larger CSAs were also associated with greater vertical stiffness and running speeds (Monte and Zamparo, 2019). Greater CSA, as opposed to a longer length, may therefore provide greater return of elastic energy, suited more to transferring force and in turn, aiding performance.

In conclusion, GM tendon mechanical and material properties do not appear to differ between elite youth male soccer players and recreationally active male controls. However, correlations between these properties and performance variables during jumping tasks suggest a less extensible tendon may facilitate power outputs during a unilateral vertical CMJ. Similarly, a stiffer and larger GM tendon correlated with higher forces generated during a bilateral drop jump and leg stiffness parameters, potentially serving as a protective mechanism. Future research should therefore seek to investigate whether the extensibility and size of the GM tendon can adapt to soccer-specific training and if that coincides with improvements in jump performance for elite youth soccer players.

Chapter Six

The effect of 12 weeks' in-season soccer training on gastrocnemius medialis muscle-tendon properties and jump performance in elite youth soccer players

Prelude

Chapters 4 and 5 provided a detailed description of the GM MTU properties in ESP, and demonstrated that certain MTU characteristics differed between ESP and CON, suggesting that habitual loading during soccer-specific training might cause chronic GM MTU adaptations. This hypothesis is tested in this final experimental chapter, which builds on the previous two chapters by investigating the effects of a period of chronic in-season soccer training on GM MTU properties and power/reactive strength during jumping tasks. Therefore, the efficacy of soccer training at eliciting adaptation of the GM MTU, and changes in jump performance, can then be evaluated. Furthermore, due to the time constraints of working with elite athletes, the simplified method of calculating GM tendon properties (as outlined in chapter 3) was used in this study chapter.

Abstract

The aim of this study was to investigate gastrocnemius (GM) muscle-tendon unit (MTU) properties and jump performance over a 12-week in-season training programme in elite male youth soccer players (ESP). Fourteen ESP (mean±SD, age = 18.8±1.4 years, height = 1.80±0.08 m, mass = 73.0±5.23 kg) from an English Premier League Category 1 football academy participated in this study. Participants completed their regular structured soccer-specific training programme (no plantar flexor-specific resistance training) over a 12-week period from the start of the playing season. Participants performed pre- and post-training assessments, which included GM anatomical cross-sectional area and architecture (thickness, fascicle pennation angle, θ_p, and length) measured via high-resolution ultrasonography at 50% GM length, and isometric plantar flexion and dorsiflexion maximum voluntary contraction via isokinetic dynamometry. The GM tendon force-elongation relationship was measured using a combination of isokinetic dynamometry, ultrasonography and electromyography. Participants also performed a series of unilateral and bilateral countermovement jumps (CMJ) and bilateral drop jumps on a force platform. GM θ_p (pre-training: 18.0±2.5°; post-training: 17.2±2.6°; P=0.038), maximal tendon elongation (pre-training: 2.68±0.58 cm; post-training: 2.30±0.46 cm; P=0.019) and maximal tendon strain (pre-training: 12.8±2.4 %; post-training: 11.1±2.5 %; P=0.019) all reduced post-training. Only unilateral horizontal CMJ projectile range changed pre- to post-training (pre-training: 0.93±0.15 m; post-training: 1.07±0.12 m; P=0.001), and this change was unrelated to changes in GM θ_p, tendon elongation or strain. Although these results suggest a 12 week period of in-season soccer training causes certain GM MTU adaptations, they do not appear to influence changes in jump performance.

6.1 Introduction

Key physical performance indicators (KPI), such as sprinting speed, have been identified previously in elite youth soccer players (Emmonds *et al.*, 2016; Murtagh, Brownlee, *et al.*, 2018). Enhancing these physical abilities requires an understanding of the physiological factors that can influence performance. Jump assessments, such as a countermovement and drop jumps, can be implemented to isolate physical qualities pertinent to these prominent KPIs (Faude, Koch and Meyer, 2012; Nagahara, Naito, Miyashiro, *et al.*, 2014; Douglas *et al.*, 2020). Our findings in chapters 4 and 5 demonstrated that relationships exist between specific gastrocnemius medialis (GM) muscle-tendon unit (MTU) characteristics and performance in these jumping tasks, suggesting that improving performance in KPIs could be influenced by altering these properties, e.g. via specific resistance exercise training. However, before supplementing an athlete's training programme with an additional exercise intervention, it is important to understand whether positive adaptation occurs primarily through participation in the sport-specific training, e.g. in-season pitch-based training in soccer players.

Few studies have monitored muscle-tendon properties during a period of sport-specific training without the inclusion of an isolated training intervention (Nimphius, McGuigan and Newton, 2012; Sponbeck *et al.*, 2017; Timmins *et al.*, 2017). Instead, cross-sectional study designs have been employed to identify differences in MTU properties between various athletic populations (Arampatzis *et al.*, 2007; Wiesinger *et al.*, 2016; Aeles *et al.*, 2017; Murtagh, Nulty, *et al.*, 2018). Typically, larger muscle volume is found in athletes with a principal requirement for speed and power (i.e. sprint cyclists, sprinters and soccer players) (Murtagh, Nulty, *et al.*, 2018; Sugisaki *et al.*, 2018; Fukutani *et al.*, 2020; Kordi *et al.*, 2020; Miller *et al.*, 2020). Additionally, longer GM muscle fascicles are found in elite athletes, who

specialise in sprinting or jumping, which appear to be beneficial for performance in those sports (Abe, Kumagai and Brechue, 2000; Kumagai *et al.*, 2000; Aeles *et al.*, 2017).

Tendon properties on the other hand, are often dependant on the intensity of loading rates caused by the sporting disciplines. Larger cross-sectional areas have been reported in athletes with high loading rates (i.e. running and soccer) (Magnusson and Kjaer, 2003; Kongsgaard *et al.*, 2005; Wiesinger *et al.*, 2016; Murtagh, Stubbs, *et al.*, 2018), whilst differences in tendon stiffness could be considered specific to the respective MTU (i.e. patellar *vs*. Achilles). Greater compliance has been reported in the patellar tendon in sprinting and soccer populations (Kubo *et al.*, 2000, 2011; Arampatzis *et al.*, 2007; Murtagh, Stubbs, *et al.*, 2018), whilst less consistent findings exist for the Achilles tendon (Kubo *et al.*, 2000, 2017; Arampatzis *et al.*, 2007; Stafilidis and Arampatzis, 2007), which may be stiffer in sprinters compared to endurance runners and untrained individuals (Arampatzis *et al.*, 2007). It is however, unclear whether these between group differences are the consequence of an adaptation to the sport-specific training stimulus, or to supplementary training (e.g. chronic resistance exercise). In particular, limited information exists pertaining to GM MTU characteristics in soccer players, despite their relationship with power and reactive strength during jumping tasks, as reported in earlier chapters (4 and 5). Moreover, it is not known how relationships between GM MTU properties and jump outputs might change in response to altered muscle-tendon properties following a period of soccer-specific training.

Previous research has documented the different stimuli required for generating adaptation of skeletal muscle and tendinous tissue (Arampatzis, Karamanidis and Albracht, 2007; Arampatzis *et al.*, 2010; Bohm *et al.*, 2014; Wiesinger *et al.*, 2015; Franchi, Reeves and Narici, 2017; Damas, Libardi and Ugrinowitsch, 2018). Few studies investigating soccer players have monitored architectural changes over a prolonged period, but those that have are conducted in the knee extensors or flexors (Coratella *et al.*, 2018; Lovell *et al.*, 2018;

Medeiros *et al.*, 2020; Mendiguchia *et al.*, 2020). However, it is currently unknown whether soccer-specific training can provide a suitable stimulus to elicit adaptation of the GM MTU.

Therefore, the aim of this study was to observe a group of elite youth soccer players over the course of a 12-week in-season pitch-based training programme (from the start of the playing season) and identify whether the GM MTU properties changed in response to training. Furthermore, we aimed to identify if any changes in jump performance were related to changes in GM MTU properties. We hypothesised that soccer-specific training would increase GM muscle size and fascicle pennation angle, and increase GM tendon stiffness, which would all contribute to increases in power output during CMJs and drop jumps.

6.2 Method

6.2.1 Participants

Fourteen elite youth male soccer players (ESP) (age = 18.8 ± 1.4 years, height = 1.80 ± 0.08 m, mass = 73.0 ± 5.23 kg) from an English Premier League Category 1 football academy were recruited, all of whom provided written informed consent to participate in this study. Ethical approval was provided by the Liverpool John Moores University Research Ethics Committee and the study complied with the Declaration of Helsinki.

6.2.2 Experimental Design

This study was designed to determine the effects of a regular in-season soccer mesocycle on the GM MTU properties and jump performance. Participants attended the laboratory on three separate occasions (familiarisation; pre-training; and post-training); familiarisation and pre-training were separated by at least 48 hours, while post-training occurred 12-13 weeks after pre-training. Attendance at the familiarisation session involved becoming acquainted with all the jump and plantar-/dorsiflexor assessments. During the pre- and post-training sessions, participants were required to arrive at least 48 hours post any intensive exercise. On each

occasion, participant height and mass were recorded, following which, participants laid prone on a therapy bed and, with their ankle fixed in a neutral position (0°), ultrasound images of the GM muscle and tendon were taken to determine muscle architecture (muscle thickness, fascicle length, L_f, and pennation angle, θ_p), muscle and tendon size (anatomical cross-sectional area), along with tendon length. With the participants seated on an isokinetic dynamometer, at least 3 plantar flexion and dorsiflexion maximal voluntary isometric contractions (MVCs) were generated. Furthermore, 6-s ramped MVCs were performed to determine the tendon force-elongation relationship, and passive ankle rotations were undertaken to determine the Achilles tendon moment arm via the tendon excursion method (synchronised measurements of tendon displacement via ultrasound and change in ankle angle). Surface EMG electrodes recorded agonist (GM) and antagonist (tibialis anterior) muscle activity during all trials. Participants were then taken through a standardised warm up, which involved jogging, dynamic movements and stretches before completing 3 practice trials of each jump type. Following this, participants completed 3 trials of each jump protocol on a force plate recording vertical, medio-lateral, and anterior-posterior ground reaction forces.

For a detailed account of the methods used to determine muscle morphology (anatomical cross-sectional area, ACSA, fascicle length, L_f, pennation angle, θ_p, and muscle thickness, MT), tendon properties (moment arm, stiffness, strain, elongation, stress, CSA, Young's modulus), and jump performance (height, projectile range, peak vertical power, peak horizontal power, vertical stiffness, reactive strength index), please refer to chapter 3. Due to time restrictions during the assessment periods, displacement of the GM tendon at the osteotendinous junction was not measured during the ramped MVC. Tendon elongation and displacement were therefore calculated using the method 2 (M2) outlined in chapter 3. This

method was shown to be reliable and to agree with method 1 (M1, the more comprehensive method for calculating tendon elongation, strain, stiffness and Young's modulus).

6.2.3 Training Period Monitoring

Participants took part in their regular structured soccer-specific training programme over the course of a 12 week period from the start of the competitive playing season (September). GM muscle-tendon measurements were taken the week prior to the start of the season. During all pitch-based sessions, participants wore an individual 10 Hz global positioning system (GPS) device (Apex, Statsport, Newry, UK) positioned on the upper back in a custom-made vest. Each participant's GPS device provided a measure of locomotion volume (recorded via total distance covered) and locomotion intensity (distances covered at velocities above 7 m/s, classified as sprinting). Within the training period, participants completed 45 ± 8 technical and tactical soccer sessions (59.9 ± 17.2 hours), 10 ± 3 competitive matches (12.2 ± 4.7 hours), 3 ± 2 active recovery sessions (1.2 ± 1.0 hours), 1 ± 2 individual soccer-specific pitch-based conditioning sessions (0.9 ± 1.7 hours) and 10.0 ± 2.7 lower limb strength sessions (7.8 ±1.9 hours). Total distance and sprint distance for the 12-week training period are presented in Table 1. For comparison, total distance and sprint distance are also presented for the 8-week pre-season period, immediately preceding the 12-week training period.

Whilst resistance training programmes varied slightly based on the individual needs and abilities of the players, lower limb strength training programmes followed consistent structures, involving an anterior-based compound lift (such as a split squat, rear foot elevated split squat, leg press), two posterior-based compound lifts (e.g. hip thrust, glute bridge, single leg Romanian deadlift), an adduction based exercise (i.e. Copenhagen) and an eccentric hamstring exercise (e.g. flywheel hamstring curl, nordic curl). Additional exercises were

introduced based on individual injury history or specific needs. Repetition and sets typically ranged between 3-4 sets with 5-8 reps per exercise, where progressive overload through a sessional increase in load was encouraged throughout the programme. Crucially, no plantar flexor-specific exercises were performed by the participants throughout this training period.

6.2.4 Statistical Analysis

All data are presented as mean and standard deviation. Normality of data was determined using a Levene's and Shapiro-Wilk test. Pre- to post-training comparisons of major variables were assessed using paired t-tests (if data were normally distributed) or wilcoxon signed rank test (if data were not normally distributed). Pre-season and in-season total distance and sprinting distance (weekly average) were also compared using paired t-tests. All statistical analyses were performed using SPSS (Version 26, IBM SPSS Inc., Chicago, USA), with statistical significance set at $P<0.05$.

6.3 Results

Weekly GPS outputs during pre-season and the observed training period are reported in Table 1 along with a weekly average, which was not significantly different between training periods.

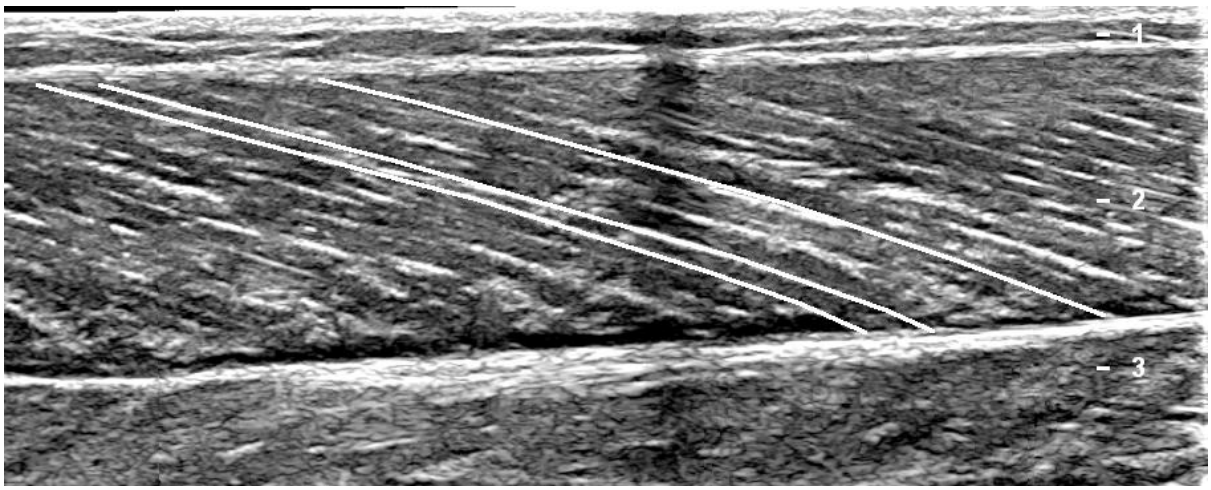
Table 1. Total distance and distance covered above 7 m/s (sprint distance) during all pitch-based sessions in pre-season and the 12 week training period. Values are mean ± SD.

Week	**Total Distance**		**Sprint Distance**	
	Pre-Season	Training Period	Pre-Season	Training Period
1	6.92 ± 0.6 km	28.6 ± 5.5 km	15 ± 47 m	219 ± 171 m
2	36.3 ± 14.9km	29.3 ± 9.5 km	181 ± 177 m	243 ± 165 m
3	25.6 ± 15.8 km	23.6 ± 6.6 km	179 ± 158 m	172 ± 149 m
4	29.7 ± 12.9 km	27.4 ± 8.6 km	152 ± 154 m	236 ± 138 m
5	25.2 ± 9.1 km	27.9 ± 7.5 km	280 ± 231 m	229 ± 157 m

6	23.7 ± 7.3 km	27.8 ± 10.9 km	150 ± 231 m	253 ± 210 m
7	33.6 ± 4.3 km	21.0 ± 12.3 km	251 ± 166 m	178 ± 160 m
8	27.8 ± 2.9km	19.8 ± 11.4 km	260 ± 131 m	135 ± 117 m
9		29.7 ± 6.7 km		225 ± 160 m
10		27.4 ± 8.0 km		206 ± 160 m
11		32.8 ± 8.6 km		264 ± 163 m
12		31.2 ± 9.1 km		267 ± 135 m
Week Average	28.8 ± 7.9 km	29.0 ± 7.6 km	184 ± 117 m	216 ± 112 m

Pre- and post-training data for morphological characteristics of the GM muscle and the GM tendon are displayed in Table 2. Apart from GM θ_p decreasing post-training, no other pre to post changes in morphological muscle-tendon variables were found.

A



B

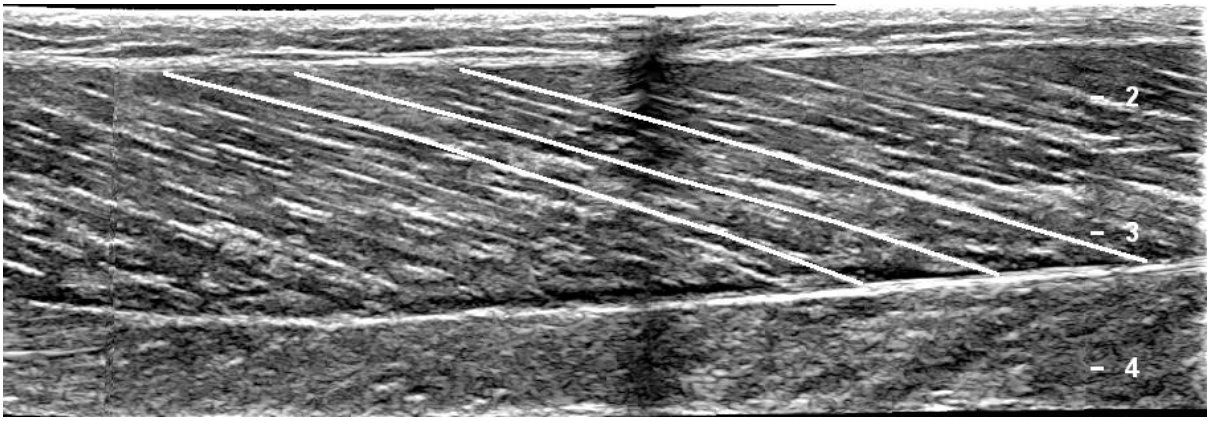


Figure 1. Representative muscle architecture ultrasound EFOV images taken at 50% GM muscle length of the same player pre- (**a**) and post-training (**b**).

Table 2. Morphological characteristics of the GM muscle and tendon before (pre) and after (post) the 12 week training period. Values are mean ± SD.

Variable	Pre	Post	P Value
GM L_f (cm)	5.90 ± 0.94	5.82 ± 1.02	0.730
GM θ_p (°)	18.0 ± 2.5	17.2 ± 2.6 *	0.038
GM ACSA (cm^2)	13.5 ± 2.0	13.5 ± 2.2	0.977
GM MT (cm)	1.74 ± 0.17	1.76 ± 0.20	0.864
Tendon length (cm)	21.15 ± 2.41	21.21 ± 2.59	0.746
Tendon CSA @ 1 cm (mm^2)	79.1 ± 0.1	77.2 ± 1.2	0.570
Tendon CSA @ 2 cm (mm^2)	70.2 ± 1.0	72.3 ± 1.3	0.401
Tendon CSA @ 3 cm (mm^2)	63.4 ± 0.1	65.5 ± 1.0	0.394
Tendon CSA @ 4 cm (mm^2)	62.1 ± 0.8	60.9 ± 1.0	0.526
Mean tendon CSA (mm^2)	68.7 ± 0.6	68.4 ± 1.0	0.848

GM, gastrocnemius medialis muscle; L_f , GM fascicle length; θ_p , GM fascicle pennation angle; *MT*, GM muscle thickness; *ACSA*, anatomical cross-sectional area.

Table 3 displays the pre- and post-training values for the GM tendon mechanical and material properties. Maximal elongation and strain were both lower post-training but no other differences were found.

Table 3. GM tendon mechanical and material properties before (pre) and after (post) the 12 week training period. Values are mean ± SD.

Variable	Pre	Post	P Value
Individual Stiffness (N/mm)	697 ± 439	698 ± 267	0.997
Common Stiffness	702 ± 380	645 ± 160	0.669
Individual Elongation (cm)	2.68 ± 0.58	2.30 ± 0.46 *	0.019
Common Elongation (cm)	1.85 ± 0.51	1.65 ± 0.24	0.166
Individual Strain (%)	12.8 ± 2.4	11.1 ± 2.5 *	0.019
Common Strain (%)	8.90 ± 2.03	7.18 ± 2.40	0.074
Individual YM (GPa)	1.62 ± 0.45	1.88 ± 0.57	0.139
Common YM (GPa)	1.64 ± 0.63	1.82 ± 0.46	0.308
Individual Force (N)	1411 ± 123	1347 ± 197	0.552
Individual Stress (MPa)	20.75 ± 4.82	17.14 ± 7.85	0.147
Common Stress (MPa)	13.45 ± 1.23	13.68 ± 1.97	0.526

YM, Young's modulus; *Individual*, maximum value for that variable; *Common*, value for that variable measured at the highest common force

For the jump parameters, only unilateral horizontal CMJ projectile range increased from pre- to post-training (Table 3).

Table 4. Jump outputs before (pre) and after (post) the 12 week training period. Values are mean ± SD.

Variable	Pre	Post	P Value
VCMJ Jump Height (cm)	43.3 ± 7.2	44.5 ± 7.0	0.470
VCMJ Peak Vertical Power (Watts/kg)	228 ± 35	240 ± 38	0.085
LVCMJ Jump Height (cm)	26.3 ± 6.1	24.0 ± 3.1	0.182
ULVCMJ Peak Vertical Power (Watts/kg)	126 ± 29	128 ± 31	0.739
HCMJ Projectile Range (m)	1.32 ± 0.16	1.37 ± 0.12	0.101
HCMJ Peak Horizontal Power (Watts/kg)	184 ± 34	182 ± 61	0.281
ULHCMJ Projectile Range (m)	0.93 ± 0.15	1.07 ± 0.12*	0.001

ULHCMJ Peak Horizontal Power (Watts/kg)	132 ± 33	141 ± 48	0.112
DJ Contact Time (s)	0.193 ± 0.013	0.193 ± 0.020	0.802
DJ Flight Time (s)	0.513 ± 0.040	0.514 ± 0.052	0.880
DJ Jump Height (cm)	32.4 ± 5.0	32.7 ± 6.4	0.781
DJ RSI (AU)	2.67 ± 0.30	2.69 ± 0.41	0.975
DJ Peak Force (N)	5288 ± 883	5077 ± 972	0.363
DJ Vertical Stiffness ($N \cdot m \cdot kg^{-1}$)	503 ± 167	492 ± 220	0.683

VCMJ, vertical countermovement jump; *ULVCMJ*, unilateral vertical countermovement jump; *HCMJ*, horizontal countermovement jump; *ULHCMJ*, unilateral horizontal countermovement jump; *DJ*, drop jump.

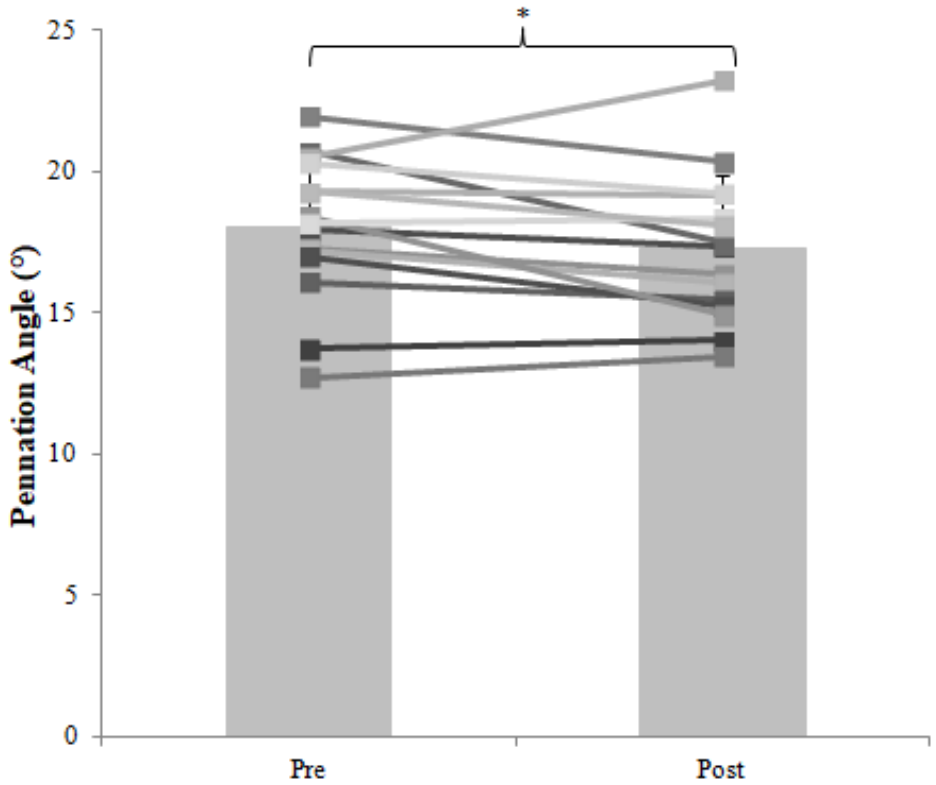


Figure 2. Individual values for pennation angle for before (pre) and after (post) the 12 week training period as well as mean ± SD. * Significant difference ($P<0.05$).

6.4 Discussion

The present study aimed to investigate the effect of a 12-week period of soccer-specific training in elite youth soccer players on changes in the morphological, mechanical, and material properties of the GM MTU, as well as changes in performance during jump

assessments. The main findings refute the hypothesis that increases in GM fascicle pennation angle and mechanical tendon properties of the GM MTU would occur post-training. The majority of muscle-tendon properties remained unaffected, whilst there were reductions in GM θ_p, maximal tendon elongation and strain. We also hypothesised that increases in vertical power output during unilateral vertical CMJ would occur in conjunction with the GM MTU morphological changes. However, no change in any jump parameter, apart from unilateral horizontal CMJ projectile range, was found from pre- to post-training.

No changes in muscle size (ACSA or MT) or L_f were found, but the reduction in GM θ_p following the 12 week training period was surprising given the findings in chapter 4, i.e. GM θ_p was larger in ESP compared to CON. The finding in Chapter 4 suggests that GM θ_p might adapt to soccer-specific training by increasing parallel-aligned sarcomeres in response to the chronic training volume/intensity, thus explaining the higher jump performance variables in ESP compared to CON. However, the findings in the present study would suggest in-season soccer training does the opposite, i.e. GM θ_p decreases, possibly by losing sarcomeres in parallel. Previously, smaller GM θ_p have been observed in high-level sprinters compared to non-sprinters (Kumagai *et al.*, 2000; Abe *et al.*, 2001), whilst sprint and jump training interventions have led to a reduction in VL θ_p (Blazevich *et al.*, 2003). These studies could suggest that smaller θ_p is a favourable adaptation for sprint and jump performance, potentially through assisting a faster contractile velocity of the whole muscle (Degens, Erskine and Morse, 2009). GM θ_p may have decreased in the current study as a response to the sprinting exposure during soccer training and matches. Individual changes in GM fascicle pennation angle from pre- to post-training period are displayed in figure 2, which highlights the individual adaptation to this training phase. Clearly, there is not an homogenous response within the group, which may represent the differing physical demands placed on individuals in various playing positions. Future research could look to examine the differing adaptive

responses to soccer training, relative to playing position. Crucially, however, this change did not correlate with the change in projectile range during a unilateral horizontal CMJ, thus suggesting it did not contribute to this increase in jump performance.

In contrast, previous research monitoring architectural changes in elite soccer players have only reported increases in L_f and θ_p of the biceps femoris long head following the addition of a training or sprint training intervention, whereby soccer training alone did not alter muscle architecture (Mendiguchia *et al.*, 2020). However, none of the participants in the current study underwent any isolated plantar flexor specific resistance training, which might explain why no increases in θ_p or L_f were observed. Furthermore, the aforementioned reductions in VL θ_p following sprint and jump training reported by Blazevich *et al.*, (2003) could instead be the effect of cessation of resistance training that was performed for 4 weeks prior to the sprint and jump training. These findings might demonstrate how different MTUs adapt to specific stimuli, but may also represent the training-specific architectural adaptations.

Following a period of detraining, muscle architecture typically shows a trajectory of reverting back to its pre-trained state with a reduced θ_p, although may still possess adaptation depending on the time frame observed (Blazevich *et al.*, 2007; Timmins *et al.*, 2016; McMahon *et al.*, 2019). In comparison to the 12 week training period monitored in the present study, the pre-season soccer training and competition period comprised similar average weekly distances covered (28.8 ± 7.9 km *vs.* 29.0 ± 7.6 km, $P > 0.05$, for pre-season *vs.* current study period, respectively), along with the distance covered above 7 m/s (184 ± 117 m *vs.* 216 ± 112 m, $P > 0.05$), and no plantar flexion resistance training was conducted in either period. Therefore, a detraining effect appears unlikely, although caution should be used when using basic GPS metrics alone to determine training load, and fluctuations in architectural parameters over the course of the training period may have occurred as a consequence of variable training load that was not evident from the GPS data.

Similar to the current study, Timmins et al., (2017) observed the temporal changes that occur to the architecture of the biceps femoris long head during an in-season period of Australian Football League. An initial increase in Lf following 5 weeks of training was followed by a slight reduction over the remaining 17 weeks in previously un-injured athletes. Muscle θp, however, appeared to decrease over the first 9 weeks before increasing over the remaining 14 weeks. Values at week 13 were smaller (moderate effect size) than initial values in previously un-injured athletes, whilst no change in muscle thickness was observed across the 23-week season. These findings may support those of the present study, specifically that fluctuations in GM muscle architecture may occur throughout the course of a competitive season, potentially influenced by alterations in training volume/intensity. Further time-points would be required to identify if such a trend occurred in the GM in the current study. It is worth acknowledging that the mean group change in fascicle pennation angle lies within the range of variability for that assessment (CV=6.4%, TE=0.94°), as reported in chapter 3. This highlights the importance of identifying the reproducibility of physiological assessment prior to any longitudinal monitoring, as the meaningfulness of the change observed in the current study may be less impactful than first thought. Nevertheless, maintaining and increasing fascicle pennation angle should form part of a supplementary in-season training programme for ESP given our findings in chapter 4 that demonstrated its association with peak vertical power during a unilateral vertical CMJ.

Similar to the morphological characteristics, limited changes in the mechanical properties of the GM tendon were found following the 12-week training period. Only a reduction in maximal elongation and strain were present, whilst all other parameters remained constant. These findings might be surprising given the limited evidence of changes in GM elongation or strain following a period of resistance training (Kubo *et al.*, 2012; Geremia, Baroni, Bobbert, *et al.*, 2018; Werkhausen *et al.*, 2018). Additionally, these findings do not coincide

with a detraining effect, as previous intervention research has reported an increase in maximal elongation of the GM tendon following the cessation of resistance training, possibly caused by a change in collagen fibre structure (Kubo *et al.*, 2012). This adaptation appears to be specific to the respective MTU, as the patellar tendon shows clear adaptive responses to loading and unloading during resistance training (Seynnes *et al.*, 2009; Kubo, Ikebukuro, *et al.*, 2010). It is unclear why this adaptation occurred in our soccer players; although one possible explanation could be a change in resting crimp patterns. Given that overall elongation reduced but no change in tendon force or stiffness occurred, the cause for altered strain is probably not due to the tendon length changes that occur over 80-100% MVC. Instead, these findings suggest that changes to the compliance of the tendon during the toe region might explain the observed change in maximal elongation, where typically, elongation in the toe region is caused by straightening of resting crimp (Maganaris, Narici and Maffulli, 2008). Research investigating changes in Achilles tendon crimp patterns following a 12 week period of running in rats has reported a reduction in central tendon regions for crimp number, along with larger and flatter fibre crimps in the proximal and distal regions, which may improve the release of the stored strain energy (Franchi *et al.*, 2013). Therefore, soccer-specific training could incur a similar adaptation, as reductions in crimp angle and number could reduce the elongation that occurs during the toe region of a force-elongation curve, which might ultimately reduce overall elongation and strain. Alternatively, the reductions in tendon elongation and strain may have been due to a change in the material properties of the tendon (e.g. increased density of collagen fibrils) that did not quite translate to changes in stiffness or modulus.

Further adaptation of the GM tendon mechanical or morphological properties might have been expected, due to previous cross-sectional findings in athletic populations (Rosager *et al.*, 2002; Magnusson and Kjaer, 2003; Wiesinger *et al.*, 2016). Larger GM tendon CSA of

runners and high-intensity loading based sports have been found in these studies, although limited data exist following in-season changes to tendon size (Sponbeck *et al.*, 2017). However, the current study found no change in size or stiffness of the GM tendon. Earlier observation-based investigations by Sponbeck *et al.*, (2017) found a gradual increase in GM tendon CSA over the course of a cross-country running season, whilst Hansen *et al.*, (2003) found no change in GM tendon CSA following a period of endurance running in previously untrained individuals. Disparity in these findings could be caused by different training regimes and overall loading, although a lack of such detail in those studies prevents further postulation.

Previous research has shown that adaptation to tendinous tissues requires a markedly different stimulus than skeletal muscle to trigger mechanical and morphological adaptation (Arampatzis, Karamanidis and Albracht, 2007; Arampatzis *et al.*, 2010; Bohm *et al.*, 2014; Geremia, Baroni, Bobbert, *et al.*, 2018; Geremia *et al.*, 2019; Schoenfeld *et al.*, 2020). Several authors have demonstrated the dependence of tendon adaptation on the magnitude and duration of the applied mechanical strain (Arampatzis, Karamanidis and Albracht, 2007; Arampatzis *et al.*, 2010; Bohm *et al.*, 2014). Increases in mechanical stiffness have been observed following 4-12 weeks of resistance training with loads >80% 1RM (Kubo, Morimoto, Komuro, Yata, *et al.*, 2007; Seynnes *et al.*, 2009; Malliaras *et al.*, 2013), which might promote changes to intrinsic material properties, rather than through the addition of collagenous tissue (Bohm, Mersmann and Arampatzis, 2015; Wiesinger *et al.*, 2015; Geremia, Baroni, Bobbert, *et al.*, 2018). Morphological changes, however, appear to possess a longer adaptive time-course (8-14 weeks) and require very high intensity of repetitive loading strategies (>90% MVC, >3s loading) to generate changes (Arampatzis, Karamanidis and Albracht, 2007; Bohm *et al.*, 2014; Geremia, Baroni, Bobbert, *et al.*, 2018). Therefore, these observations might explain those of Chapter 5, which displayed no differences in GM

tendon mechanical properties between a group of ESP and CON participants. Again, these findings suggest that soccer-specific training is not enough of a stimulus to generate the adaptations that typically occur following a period of high-intensity resistance training.

A second aim of this investigation was to identify if any changes to physical KPI assessments took place following 12 weeks of soccer-specific training in ESP. The majority of jump parameters remained unaffected by the training period, and only unilateral horizontal CMJ projectile range was found to increase from pre- to post-training. This could support our findings from chapter 4, which demonstrated a significant difference between a group of ESP and CON participants for unilateral horizontal CMJ projectile range. The ESP group had a greater projectile range, which suggested either a training-induced improvement in jump performance or a genetic predisposition that enables an augmented performance in jumping tasks.

However, no correlations were found between any GM MTU characteristics and unilateral horizontal CMJ projectile range. Instead, previous research has reported correlations between the same jump parameter and the elongation, strain and Young's modulus of the patellar tendon (Murtagh, Stubbs, *et al.*, 2018). This could suggest that the muscle-tendon properties of the knee extensors, known to be more favourable in ESP (Murtagh, Stubbs, *et al.*, 2018), are more influential for unilateral horizontal jump performance than the plantar flexor MTU. Therefore, due to the emphasis in the present study of the resistance training on the musculature around the knee and hip (rather than the plantar flexors), it is possible that adaptations occurred in this MTU (rather than being due to any changes in the GM MTU), which altered these properties and improved jumping projectile range. Although without isolated examination of these properties, this hypothesis remains speculative.

Whilst these findings might suggest in-season improvements in physical KPI assessments can be attained, there are conflicting findings from another study (Brownlee *et al.*, 2018). This study monitored changes in neuromuscular strength of ESP following an 8-week in-season training period, and found no change in isometric mid-thigh pull strength and no difference in comparison to a control population. These findings support those from the present study, which suggest that an academy soccer programme does not provide an adequate stimulus to generate significant improvements in neuromuscular strength. Similarly, (Gil-Rey, Lezaun and Los Arcos, 2015) reported trivial changes to CMJ jump height following a 9 week in-season training cycle in elite youth soccer athletes of similar age to those in the current study. In conjunction with the present study, these findings suggest that without supplementary resistance training interventions (targeting specific muscle groups), soccer-specific training (solely pitch-based training) is not a sufficient stimulus to generate changes in physical KPIs.

In conclusion, a 12-week period of soccer training does appear to cause some adaptations in the GM MTU but these do not seem to influence changes in jump performance. Reductions in GM fascicle pennation angle may be attributed to the exposure of sprinting within soccer training and matches, possibly to facilitate a faster shortening of the overall muscle. Alternatively it may represent an adaptation and detraining effect in response to a lack of progressive overload in-season for the plantar flexors. In addition, GM tendon elongation and strain reduced post-training, which might be attributed to a change in resting crimp angle, or an increase in collagen fibril density as a consequence of habitual loading. Future research should seek to determine whether plantar flexor resistance training interventions, concurrent to soccer training, can elicit greater adaptation of the GM MTU, and whether this can improve performance in jump assessments.

Chapter Seven

Synthesis of Findings

7.1 Synthesis

The purpose of this chapter is to provide a logical interpretation of the major findings from each experimental chapter in relation to the aims and objectives of the PhD thesis. This chapter will discuss the limitations associated with collecting GM muscle-tendon property data *in vivo*, and will provide direction for future research, addressing these restrictions. It will also provide practical applications regarding the assessment of lower body power and stiffness alongside potential training interventions for the GM MTU in elite youth soccer players aiming to augment these physical qualities.

The aim of this thesis was to investigate the muscle-tendon properties of the GM MTU in a cohort of elite youth soccer players (ESP) and understand how these characteristics influence lower body power and reactive strength. Four main objectives were implemented to achieve this aim, and these objectives were reached in the various chapters within this thesis:

1) Determine the reproducibility of current assessment methods used to quantify: lower body power and reactive strength capabilities; morphological characteristics of the GM; mechanical and material properties of the GM tendon. This objective was met in chapter 3.
2) Identify if GM muscle characteristics or performance in jumping tasks could distinguish a group of ESP from control individuals (CON). This objective was achieved in chapter 4.
3) Establish whether GM tendon properties differed between ESP and CON, and whether these properties were related to lower body power and reactive strength. This objective was reached in chapter 5.
4) Determine the effect of 12 weeks' in-season soccer training on GM MTU properties and jump performance in ESP. This objective was met in chapter 6.

7.2 General discussion

Lower body power and reactive strength qualities are two prominent determinants of acceleration and maximal velocity sprinting performance, which are known KPIs in soccer (Faude, Koch and Meyer, 2012; Emmonds *et al.*, 2016; Colyer *et al.*, 2018; Murtagh, Brownlee, *et al.*, 2018; Douglas *et al.*, 2020). Isolation and quantification of these physical qualities can help practitioners profile soccer athletes and allow individualised training interventions to be applied based on athlete ability. Jump assessments, such as a CMJ and DJ, have been frequently used to provide an objective assessment of both power and reactive strength (Meylan *et al.*, 2010; Maloney *et al.*, 2016; Murtagh *et al.*, 2017; Douglas *et al.*, 2020). The specificity, and calculation method, of a bilateral vertical CMJ to determine power has however, been questioned previously (Samozino *et al.*, 2008; Murtagh *et al.*, 2017; Morin *et al.*, 2019). Instead, authors have suggested unilateral, horizontal jumps can provide improved, more appropriate, sport-specific assessment of power (Murtagh *et al.*, 2017). However, methods of doing so are infrequently utilised and limited reliability data exist regarding these alternate methods.

Findings from chapter 3 demonstrate that peak power in the direction of the jump (i.e. vertical power with a vertical CMJ and horizontal power with a horizontal CMJ) could be calculated reliably. Reproducibility for these peak power measurements were improved during bilateral jumps compared with their unilateral counterparts. Our findings do support previous reports that peak concentric power can be calculated reliably in the direction of the jump (Meylan *et al.*, 2010). Interestingly, vertical peak power during horizontally orientated CMJs had a very high CVs (26-33%) and RLoAs (±40-84%) for both unilateral and bilateral jumps. Therefore, we recommend that peak vertical power should not be monitored during horizontal CMJ due to the large variability associated with it. Furthermore, our findings from chapter 3 suggest that only horizontal CMJ can differentiate ESP from a control population.

This is in contrast with previous research that investigated vertical power in a different cohort from the same population, reporting peak vertical power during unilateral, vertical, horizontal and medial CMJ could distinguish ESP from non-elite soccer players (Murtagh *et al.*, 2017). Although no differences in any variables during vertical CMJ were found between ESP and CON in the current thesis, the poor reproducibility of these variables (highlighted in Chapter 3), and the higher performance capability in CON (compared to non-ESP in Murtagh *et al*., 2017), might explain these discrepancies between studies. Instead, horizontal peak power during a bilateral or unilateral horizontal CMJ might provide improved, more appropriate assessment of lower body power in soccer players, due to its relatively high reproducibility and ability to distinguish between ESP and CON/non-ESP, and should be utilised in future studies.

Similar to CMJ, several variables are frequently utilised for identifying reactive strength qualities during a DJ. RSI and lower limb stiffness have been examined previously, often showing similarities and a close relationship (Kipp *et al.*, 2018). However, in chapter 3, we outlined that RSI can be influenced by flight time and airborne technique, whilst leg stiffness is solely determined by kinetic parameters during ground contact. Therefore, leg stiffness could provide a more informative assessment of an individual's ability to apply large forces and resist deformation. However, both assessments displayed slightly larger than desirable variability through CVs (10.2-18.7%) and RLoAs (±39-65%). Whilst CVs from the current thesis were comparable to previous reports of these measures (Feldmann *et al.*, 2012; Byrne *et al.*, 2017; Douglas *et al.*, 2018; Maloney, Richards and Fletcher, 2018), the variability in contact time might explain a significant amount of discrepancy between testing occasions for RSI and leg stiffness. Both RSI and leg stiffness can be influenced by contact time; whilst no significant difference was present between contact time times between testing occasions, any variance incurred would therefore provide a knock-on effect on the proceeding reactive

strength assessment. Whilst the group means for RSI and leg stiffness appeared to be higher in ESP compared to CON, they were not significantly higher. A previous investigation has, however, utilised reactive strength qualities to differentiate between different athletic populations (Douglas *et al.*, 2018).

Performance assessments, such as CMJs and DJs, demonstrate the power and reactive strength capabilities of the individual athlete. However, the specific characteristics of the respective MTUs of the hip, knee and ankle are thought to underpin function in these tasks. Several authors have outlined the importance of the ankle, in particular the plantar flexors, for performance in both CMJs and DJs (Bobbert, Huijing and van Ingen Schenau, 1986b; Pandy and Zajac, 1991; Farley and Morgenroth, 1999; Kuitunen, Ogiso and Komi, 2011; Maloney *et al.*, 2016). Behaviour of the contractile tissue (GM) and elastic tissue (GM tendon) show distinctive behaviours whilst jumping, where the muscle is required to generate large forces during a near isometric contraction and the tendon is responsible for ensuring a rapid recoil of the MTU can be achieved via a catapult action (Kurokawa, Fukunaga and Fukashiro, 2001; Kurokawa *et al.*, 2003; Ishikawa, Niemelä and Komi, 2005; Sousa *et al.*, 2007). It might be expected that different mechanical and morphological properties of the GM MTU could affect its behaviour *in vivo*, which in turn could influence performance in jumping tasks.

Assessment of GM morphology can be conducted with high levels of reliability, as demonstrated in chapter 3. Our reproducibility values are in line with previous reports (Mohagheghi *et al.*, 2007; Legerlotz, Smith and Hing, 2010; Raj, Bird and Shield, 2012; McMahon, Turner and Comfort, 2016), although this is the first study demonstrating that the size and architecture in the GM can be measured using EFOV ultrasound reliably. Chapter 4 examined the relationship between GM morphology with lower limb power and reactive strength qualities. Our findings demonstrated positive correlations between GM muscle CSA and thickness with peak horizontal power during a bilateral CMJ. Furthermore, positive

relationships were found between GM fascicle pennation angle with peak vertical power and jump height in unilateral vertical CMJ, whilst GM fascicle length correlated inversely with the same jump parameters. Our findings do support those of previous studies, who reported similar observations in the gastrocnemius with positive correlations between GL muscle size and fascicle pennation angle with jump performance (Earp *et al.*, 2010; Dobbs, Nicholas, *et al.*, 2015). When considered in conjunction with its role during jumping tasks, remaining near-isometric to generate large forces, these findings suggest that a greater number of GM parallel-aligned sarcomeres would allow more force to be produced during a CMJ, which in turn would increase horizontal power output. Similarly, larger fascicle pennation angles would be indicative of a greater number of parallel-aligned sarcomeres (Wickiewicz *et al.*, 1983). Again, this arrangement of contractile tissue might favour the force-generating capacity of the GM, which in turn, could increase plantar flexion force during CMJ. An augmentation in force production would likely transfer to increasing vertical peak power, as demonstrated during unilateral vertical CMJs.

Whilst these findings might suggest that practitioners should seek to elicit hypertrophy and increases in GM fascicle pennation angle through training interventions in soccer players, it is important to first understand if the sport itself induces morphological adaptation. Interestingly, the only difference in GM morphology between ESP and recreationally active controls was a larger fascicle pennation angle in the ESP. The lack of other differences between groups could be surprising given previous investigations demonstrating longer GM fascicle lengths of track and field athletes compared to untrained individuals. (Abe, Kumagai and Brechue, 2000; Aeles *et al.*, 2017). It is, however, unclear whether the previously observed differences are caused by adaptation to sport-specific training practices, or the result of supplementary training interventions (Blazevich *et al.*, 2003). Blazevich *et al.*, (2003) demonstrated increases in fascicle length and reductions in fascicle pennation angle following

a period of sprint and jump training, although this could in part coincide with a detraining effect following the cessation of resistance training.

Regardless, interpreting findings from cross-sectional research can be misleading as it is unclear whether the sport-specific training is responsible for MTU adaptation or whether it stems from supplementary training practices. Therefore, in chapter 6 we chose to observe if morphological properties of the GM would be affected by 12 weeks' in-season academy soccer training. Whilst this programme did involve resistance training, no direct training of the plantar flexors occurred. A significant reduction in fascicle pennation angle was found post-training. Similar fluctuations of pennation angle have been reported elsewhere during an in-season training period (Timmins *et al.*, 2017), which might demonstrate continual architectural remodelling of skeletal muscle in elite athletes. Alternatively, and possibly more likely, this could represent the variation associated with the assessment of fascicle pennation angle. Researchers and practitioners should be aware that measuring GM fascicle pennation angle has a CV of 6.4%, therefore changes should only be considered meaningful if they are >6% (the present observed decrease was -4.5%). Regardless, pitch-based soccer training clearly does not evoke increases of GM fascicle pennation angle in-season.

However, as outlined in chapter 2, the contractile element is only one aspect of the GM MTU. The attaching SEE also functions to influence the transmission of force and overall recoil velocity. Chapter 5 examined the relationship between mechanical and material properties of the GM tendon with jump performance outputs. Peak vertical force and lower limb stiffness during the drop jump correlated positively with stiffness over a common force range, peak plantar flexion force and mean tendon CSA. Conversely, stress over a common force range correlated inversely with the same jump parameters. Similar findings have been reported previously, as authors have reported an apparent advantage of a stiffer GM tendon

regarding drop jump performance (Hirayama *et al.*, 2017; Abdelsattar, Konrad and Tilp, 2018).

Our findings could suggest that an inherently stiffer tendon, with a larger CSA is capable of tolerating higher force produced by the plantar flexors during a DJ. Given that leg stiffness is determined by peak GRF and centre of mass displacement, an improved ability to either tolerate and generate higher forces, or resist deformation through a shorter contact time, would appear beneficial. Hirayama *et al.*, (2017) demonstrated that increases in GM tendon stiffness enable GM muscle fascicles to contract more isometrically rather than concentrically during a drop jump, which in turn would allow for greater force production. Similarly, Abdelsattar, Konrad and Tilp, (2018) found positive correlations between GM tendon stiffness and drop jump contact time, however the current thesis is the first investigation to correlate tendon properties with markers of reactive strength.

Unlike chapter 4, where GM muscle fascicle pennation angle was larger in ESP compared to CON, no between group differences for any GM tendon mechanical or material property were reported in chapter 5. Previous studies comparing GM tendon properties between different athletic populations have reported increases in tendon CSA in those with supposedly higher habitual loading patterns (Rosager *et al.*, 2002; Magnusson and Kjaer, 2003; Wiesinger *et al.*, 2016). These previous populations typically included endurance runners and ski jumpers, which have markedly different physical demands to soccer players. For example, an endurance runner may provide repetitive loading to the GM tendon at a consistent, sub-maximal magnitude, whilst a ski jumper may undergo fewer loading cycles of the tendon, but at a much higher intensity. Soccer training and competition on the other hand, provides an exposure to a variety of movement demands, involving low intensity locomotion interspersed by periods of high-intensity accelerations, decelerations, jumps and sprints, which will result in an inconsistent loading pattern of the tendon (Akenhead *et al.*, 2013; Murtagh *et al.*, 2019).

From the findings of chapter 5, it appears that soccer training might not provide an adequate stimulus to elicit changes in GM tendon properties.

However, following a 12-week in-season training period, the findings from chapter 6 showed a significant reduction in maximal tendon elongation and strain. Periods of tendon loading frequently generate changes in mechanical stiffness, therefore, whilst these results do not coincide with the adaptation observed following a period of resistance training/detraining (Kubo *et al.*, 2012; Geremia, Baroni, Bobbert, *et al.*, 2018), they are indicative of an adaptation to training load. For example, these changes may have been due to a change in the material properties of the tendon (e.g. increased density of collagen fibrils) that did not quite translate to changes in stiffness or modulus.

In addition to these morphological and mechanical parameters, chapter 6 investigated the changes in jump performance following 12 weeks of training. An increase in unilateral horizontal CMJ projectile range was found, which could represent the effect of the supplementary resistance training programme. Given that the changes in GM MTU properties seen in the current study did not correlate with the change in unilateral horizontal CMJ projectile range, and no GM MTU property was related to unilateral horizontal CMJ projectile range in chapters 4 or 5, the specific GM muscle-tendon characteristics likely have little to no influence on this variable (or change in variable). Instead, previous research might demonstrate the importance of the knee extensor MTU on horizontal jump performance (Murtagh *et al.*, 2018). Given the lack of plantar flexor resistance training in chapter 6, but a greater focus on the musculature surrounding the knee and hip, improved performance in the unilateral horizontal CMJ could be attributed to changes in the MTU of the quadriceps femoris. However, without direct measurement of these MTU properties in the current thesis, we are reliant on previous cross-sectional research (Murtagh *et al.*, 2018).

7.3 Conclusion

To conclude, the findings from this thesis demonstrate that lower limb physical qualities and physiological characteristics can be measured reliably utilising the methods undertaken. Although there are some tentative signs of adaptation, it does not appear that in-season soccer training (in the absence of muscle-specific resistance training) provides a strong stimulus for positive adaptation of the GM MTU. Whilst it may not be appropriate to prescribe direct training interventions based on this research, certain physiological characteristics of the GM MTU do appear favourable to lower limb power and reactive strength qualities by facilitating force production. Therefore, S&C practitioners in elite soccer may wish to monitor the GM MTU properties of their players, together with the KPIs assessed in this thesis, to provide a more informative assessment of their players' physical performance (i.e. changes in GM MTU properties may explain changes in KPIs).

7.4 Limitations and future research

The current thesis provides novel information regarding the plantar flexor muscle physiological characteristics and power and reactive strength capabilities of elite youth soccer players. Through achieving our overarching aim and objectives, we have acknowledged some limitations within the specific chapters and identified areas for future research. This section aims to address the limitations of the present work and the recommendations for future research in relation to each chapter.

Suggestions arising from chapter 3

The reproducibility of power outputs during vertical and horizontal CMJ was outlined within chapter 3. Future research should utilise the same approach to collect GRF data and calculate horizontal power during horizontal CMJ. This could be more informative when comparing physical abilities between different athletic populations and monitoring the effect of training

interventions. Furthermore, given the perfect correlation between impulse and jump height (Winter, 2005), researchers and practitioners may wish to consider this force-time parameter when assessing physical capabilities. Impulse can provide a quantifiable measure of the strategy used by an individual to generate a maximal jump. Using this can improve understanding of how differences in MTU properties of the lower limb can influence and alter jump performance via a change in movement strategy. In addition, mechanical properties of the GM tendon can be calculated reliably using a combination of ultrasonography and dynamometry, however, consensus should be reached among experts in this area, to ensure consistent methods are used that will allow between-study comparisons. In particular, direct measurement of the distal displacement at the OTJ (in addition to the MTJ) to calculate overall elongation can be assessed reliably and should continue to be implemented. However, when solely assessing change over time, a quicker method without identifying OTJ displacement can provide a suitable and reliable alternative.

Suggestions arising from chapters 4 and 5

In chapters 4 and 5, the morphological and mechanical properties of the GM MTU were investigated, and how these related to jump performance. Future research should continue to utilise power and reactive strength outputs rather than jump height or distance achieved when correlating with muscle-tendon characteristics. To fully understand the influence these characteristics may have on power or reactive strength abilities, training interventions targeting these MTU properties should be implemented. Through observing how changes in MTU properties may occur in conjunction with power and reactive strength outputs, a true insight can be gained into the influential nature of these functional characteristics.

Suggestions arising from chapter 6

In chapter 6, the changes in muscle-tendon properties in ESP over a 12-week in-season period were observed. Whilst no differences were found in these properties between ESP and control individuals in previous chapters, the absence of a control group in chapter 6 might limit the significance of the findings in this chapter. Postulations regarding the observed reductions in GM muscle fascicle pennation angle, tendon elongation and strain in ESP would require the inclusion of a control group to corroborate these findings. Furthermore, future research should monitor these properties over the course of a longer period in combination with a control group. Through capturing the entirety of a season (pre-season, in-season, off-season), the adaptive nature of the MTU to different loading strategies might be better understood. Training interventions can then be supplemented at specific time points throughout the season to improve specific KPIs.

7.5 Practical applications

When profiling the physical capabilities of ESP, practitioners should utilise unilateral and bilateral horizontal CMJ and bilateral vertical DJs to assess power and reactive strength qualities. GRF must be recorded during these jumping tasks to accurately calculate horizontal power and lower limb stiffness, although several familiarisation sessions may be required to ensure adequate reproducibility, particularly for ground contact time. GM MTU properties can also be calculated reliably with sufficient training and practice of relevant techniques, whereby simpler methods can be utilised if time is limited with athletes. These properties could be measured to help explain why certain KPIs may have improved/deteriorated, and whether specific MTU overloading strategies are successful or not and why.

Practitioners can utilise this information, knowing that soccer training alone is not a sufficient stimulus to generate sufficient adaptation in the GM MTU to improve particular KPIs. Training programmes must therefore be supplemented with specific training interventions targeting the previously highlighted morphological and mechanical properties of the GM

MTU in an attempt to improve lower body power and stiffness. Resistance training strategies could be applied to increase plantar flexion strength; elicit hypertrophy of the GM with greater fascicle pennation angles; and increase size and stiffness of the GM tendon. These adaptations would likely aide lower limb power and stiffness through an increased ability to generate force, whilst a thicker, stiffer tendon could facilitate more storage and release of elastic energy, alongside tolerating higher GRF.

The required stimuli for generating hypertrophy of skeletal muscle and tendinous tissue are markedly different, as a mixture of high- and low-load training strategies can increase CSA of skeletal muscle. However, tendons require very high-intensity approaches using near-maximal isometrics or supramaximal eccentric loads, to increase stiffness and CSA. Due to the adaptive time-course for tendon, progressive overload must be applied to prevent a plateau in the adaptation, which may require manipulation of load. Plyometric training strategies, utilising short contact times and a specific focus on the plantar flexors could also be implemented in conjunction with resistance training to augment tendon stiffness and muscular activation strategies, which in turn should improve drop jump performance.

However, it is important to acknowledge that the remit of this thesis was not to stipulate the appropriateness of specific training interventions for youth soccer players. Multiple factors contribute to power and reactive strength qualities, altering one characteristic may occur at the expense of another, or even be to the detriment of another physical quality. Profiling these muscle-tendon properties longitudinally over time will help inform their adaptive response to training and how they truly influence physical performance.

Chapter Eight

References

8.1 References

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